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ON THE TAXONOMY AND ULTRASTRUCTURE OF THE FOSSIL DIATOM *GOMPHONEIS CANTALICA*

John P. KOCIOLEK* and E.F. STOERMER**

ABSTRACT. — Previous reports on the valve structure and taxonomy of the diatom *Gomphoneis cantalica* (Brun & Hérib.) M. Schm. are compared to observations made on isotype material with light and scanning electron microscopy. In particular, the number and position of the longitudinal lines are investigated and shown to be formed by internal siliceous expansions, similar to those observed in other *Gomphoneis* species. *Gomphoneis cantalica* is shown to also possess a marginal lamina which extends internally from approximately the valve margin to the edge of the valve mantle. Chambers are formed between the outer wall and lamina. Additional valve features are illustrated with the SEM. Despite having striae composed of single rows of puncta, *G. cantalica* appears ultrastructurally most similar to typical *Gomphoneis* species, thus seems best placed in this genus.

RÉSUMÉ. — Des observations antérieures sur la structure de la valve et sur la taxonomie de la diatomée *Gomphoneis cantalica* (Brun et Hérib.) M. Schm. sont comparées avec celles faites sur des isotypes en microscopie photonique et électronique à balayage. En particulier, le nombre et la position des lignes longitudinales sont étudiées et identifiées comme étant formées par des expansions siliceuses internes, semblables à celles observées chez les autres espèces de *Gomphoneis*. Il est également montré que *Gomphoneis cantalica* possède une lamina marginale qui s'étend, sur la face interne, du bord de la valve à la marge à la courbure face valvaire-manteau. Des chambres existent entre la paroi externe et la lamina. Des caractères additionels de la valve sont illustrés à l'aide du microscope électronique à balayage. Bien qu'ayant les stries composées de rangs simples de ponctuations, *G. cantalica* apparaît, du point de vue ultrastructural, comme très semblable aux espèces typiques du genre *Gomphoneis* et doit donc être placé dans ce genre.

KEYS WORDS : *Gomphoneis cantalica*, fossil diatom, taxonomy, ultrastructure, Miocène, Cantal (France).

INTRODUCTION

Previous light microscopic observations on the diatom *Gomphoneis cantalica* (Brun & Hérib.) M. Schm. (1899) have led to differing interpretations, concerning both its valve construction and its taxonomic position. Described from

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Contribution Number 438 of the Great Lakes Research Division.

upper Miocene deposits of Aurillac, Cantal, France, this species was originally placed in the genus *Gomphonema* C. A. Agardh (1824), and distinguished from other species on the basis of its large size, striae of single rows of puncta and shadow lines running longitudinally on either side of the axial area (BRUN & HÉRIBAUD *in* HÉRIBAUD, 1893). In the next year CLEVE (1894) erected the diatom genus *Gomphoneis*, in which he included large *Gomphonema* species that possess longitudinal lines and striae composed of double rows of puncta. At that time CLEVE (1894, p. 190) commented that *G. cantalicum* was not to be included in his new genus, «... as the striae are composed of simple rows of puncta». In his discussion of this species Cleve noted a single longitudinal line on each side of the axial area, located midway between the raphe and margin. SCHMIDT (1899) later made the new combination *Gomphoneis cantalica* (Brun & Hérib.) M. Schm., apparently being of the opinion that the feature of longitudinal lines shared between *G. cantalica* and Cleve's *Gomphoneis* speceis was more indicative of relationships than puncta number in the striae. Recently DAWSON (1974) and others have expressed the opposite view, that the number of puncta comprising the striae may be important in indicating relationships, by transferring doubly-punctate species of *Gomphonema* that possess no longitudinal lines to *Gomphoneis*.

The longitudinal lines of *G. cantalica*, as depicted by SCHMIDT (1899), appear to be represented by one line on either side of the axial area, found near mid-valve (as indicated by Cleve). Illustrated also is a highly thickened valve outline (SCHMIDT, 1899). SCHMIDT (1899) suggested chambers associated with the longitudinal lines appeared similar to those found in some *Pinnularia* Ehrenberg (1841) species. In his review of diatoms which possess longitudinal lines, HUSTEDT (1935) arrived at the same conclusion as Schmidt concerning the classification of *G. cantalica*. Unlike Schmidt, HUSTEDT (1935, fig. 18) noted the presence of two longitudinal lines on either side of the axial area, and depicted them closely placed to one another in the central part of the valve.

These results indicate differences in evaluation of both the number and position of the longitudinal lines, and the taxonomic status of *Gomphoneis cantalica*. The present report utilizes light microscopy (LM) and scanning electron microscopy (SEM) to reinvestigate valve features of *G. cantalica*, providing ultrastructural information to aid interpretation of light microscopic observations and help clarify the classification of this enigmatic species.

MATERIALS AND METHODS

Isotype material of *Gomphoneis cantalica* (Brun & Hérib.) M. Schm. was provided by Dr. Charles W. Reimer, from the Boyer Collection of the Academy of Natural Sciences of Philadelphia (Boyer Misc. Mat. Box 4, 161, «France Diatomées fossiles d'Auvergne Dépot d'Aurillac Murat (Cantal) Miocene Supérieur from Héribaud coll. leg. Fr. Arsène. Cleaned 11 July 1920, H.C. Wheeler, Montreal»). Dried material was suspended in distilled water and air-dried onto

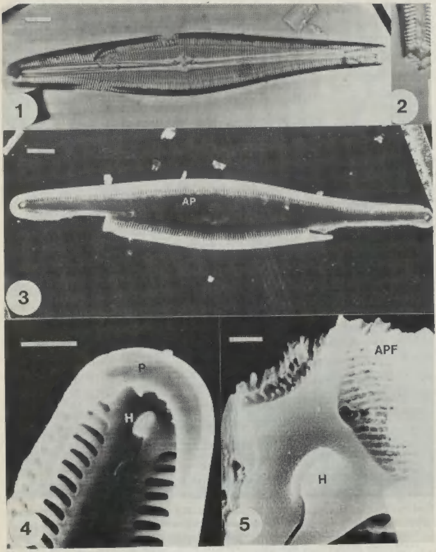

Plate I : *Gomphoneis cantalica*. — Fig. 1, 2. Light Microscopy. Fig. 1. Valve view, illustrating valve outline, filamentous raphe and position of longitudinal lines (arrows). Scale bar : 10 µm. Fig. 2. Bilobed APF with distal raphe end curving between lobes. Scale bar : 10 µm. Fig. 3-5. SEM, Internal views. Fig. 3. Valve showing structure dominated by expanded axial plate (AP). Also evident are numerous interstriae and helictoglossae at the poles. Scale bar : 10 µm. Fig. 4. Headpole, with lip-like helictoglossa (H) and pseudoseptum (P). Scale bar : 5 µm. Fig. 5. Footpole, with helictoglossa (H) and cross-shaped axial area delimiting bilobed apical pore field (APF). Scale bar : 1 µm.

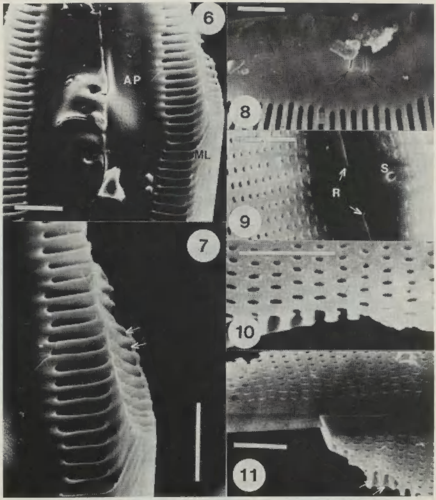

Plate 2 : *Gomphoneis cantalica.* — All figures SEM. Fig. 6-8, Internal views, fig. 9-11, External views. Fig. 6. View of valve fragment, showing expanded axial plate (AP), interstriae, marginal lamina (ML) and central nodule with stigma opening and recurved proximal raphe ends. Scale bar : 5 µm. Fig. 7. View of valve margin, showing chambers (arrows) formed between outer wall and internal marginal lamina. Scale bar : 5 µm. Fig. 8. Central nodule with recurved raphe ends and two slit-like stigmata openings (arrows). Scale bar : 5 µm. Fig. 9. Central area showing slightly enlarged raphe ends (R) and rounded stigma opening (S). Also visible are the ellipsoidal puncta. Scale bar : 5 µm. Fig. 10. Striae formed of ellipsoidal puncta. The puncta, visible externally and in cross section appear without occlusions. Scale bar : 5 µm. Fig. 11. Transverse section of raphe illustrating lock and key arrangement. Chambers (arrows) are formed between the outer wall and underlying axial plate. Scale bar : 5 µm.

glass coverslips. For light microscopy, coverslips were mounted in Hyrax and viewed with a Leitz Ortholux light microscope outfitted with brightfield optics. For scanning electron microscopy, coverslips were mounted onto aluminium stubs and sputter-coated with approximately 20 nm of gold or gold-palladium. Gold coated material was viewed on a ISI Mini-SEM, while gold-palladium coated material was viewed on a ISI DS-130. Operating voltages used were 10-15 kV.

RESULTS

Although specimens of *G. cantalica* of the material investigated were fragmented, valves appeared lanceolate-clavate in shape, tapering slightly from mid-valve to rounded poles (Fig. 1). Valve dimensions were estimated to range from 140 to 190 µm in length, and from 20 to 28 µm in breadth. Striae number 9 to 10 in 10 µm throughout the length of the valve, and are parallel in the middle portion of the valve, but become radiate at the ends. Puncta number 14 to 15 in 10 µm in each stria. Light microscopic observations indicate a highly thickened valve margin (Fig. 1). The raphe is filamentous and laterally expanded, with distal raphe ends curving opposite the side of the valve containing the stigma (Fig. 1). The apical pore field (APF) is bilobed, with the distal raphe end curving between the lobes (Fig. 2). Longitudinal lines are evident at the central part of the valve (Fig. 1).

A wide axial plate (Fig. 3) is the most striking internal valve feature shown with SEM. The axial plate appears widest at the middle portion of the valve and tapers towards the poles. Visible also is the central nodule and numerous interstriae (*sensu* ROSS et al., 1979) which are oriented perpendicular to the axial plate (Figs. 3, 6). Labiate-like helictoglossae are located at both the headpole and footpole (Figs. 3, 4; 5, respectively). Also noted at the headpole is a short pseudo-septum (Fig. 4). At the footpole the axial area becomes cruciate, forming a divided APF (Fig. 5). From the axial area the axial plate expands towards the valve margin, terminating at a distance just greater than half way to the margin (Fig. 3, 6). Chambers are formed between the axial plate and overlying outer portion of the wall. Beyond the axial plate laterally, narrow interstriae are evident and directed toward the margin (Figs. 6-8). Just prior the margin, the interstriae coalesce to form a thickened marginal lamina (Figs. 6, 7). As with the axial plate, chambers are formed between the outer wall and the lamina (Figs. 4, 6, 7). At the central nodule the raised, slit-like opening of the stigma, and recurved proximal raphe ends are present (Fig. 6, 8). One (Fig. 6) or two (Fig. 8) stigmata openings may be present.

Externally, puncta appear as elongate holes, without any apparent external flaps (*sensu* MANN, 1981) or occlusions (Figs. 9-11). Proximal raphe ends that appear slightly widened, and a rounded stigma are located in the central area (Fig. 9). Transapical views of the puncta near the axial area show chambers formed between the valve face, interstriae and axial plate (Figs. 10, 11). A trans-

apical view of the raphe indicates it is of the key and slot type (*sensu* KRAMMER, 1982a) (Fig. 11).

Girdle bands of any kind, either attached to or detached from valves or valve fragments could not be identified.

DISCUSSION

LM and SEM observations presented here are in close agreement with the dimensions and general valve shape described by Brun and Héribaud (*in* HÉRIBAUD, 1893), CLEVE (1894) and SCHMIDT (1899) for *G. cantalica*. These observations also suggest a close relationship between *G. cantalica* and classical *Gomphoneis* species. Shared features include a prominent central nodule, one or more stigmata, recurved proximal raphe ends, presence of a pseudoseptum at the headpole lip-like helictoglossae at both poles and longitudinal lines formed by an axial plate. All of these features have been previously demonstrated for *G. mammilla* (Ehrenb.) Cleve (1894) (KOCIOLEK and ROSEN, 1984). The feature of a marginal lamina, present in *G. cantalica*, was not discussed or shown for *G. mammilla* (KOCIOLEK and ROSEN, 1984), however recent reinspection of *G. mammilla* indicates a marginal lamina is present (pers. obs.). The apparent unoccluded nature of the puncta, being without external or internal flaps is shared by *G. mammilla* (KOCIOLEK and ROSEN, 1984), as well as *Gomphonema olivaceum* (Lyngb.) Kütz. (HELMCKE and KRIEGER, 1953; DRUM, 1969; DAWSON, 1974; GERMAIN, 1981), *G. quadripunctatum* (Østr.) Wisl. (DAWSON, 1974), *G. tetrastigmatum* Horikawa and Okuno (OKUNO, 1974) and *G. olivaceoides* Hust. (GERLOFF and HELMCKE, 1977; CARTER and BAILEY-WATTS, 1981), and is unlike that found in *G. parvulum* (Kütz.) Kütz (1849) (DAWSON, 1972), *G. gracile* Ehrenb. (1838) (SCHOEMAN et al., 1984), and similar *Gomphonema* species. Absence of identifiable girdle bands negates comparison with SCHMIDT's (1899, Fig. 2) observation of an unornamented girdle region in *G. cantalica*. Almost all gomphonematoidean (*Didymosphenia* M. Schm. (1899), *Gomphonema*, *Gomphoneis*, *Rhoicosphenia* Grunow) species have been shown to possess punctate girdle bands. Based on the ultrastructural features discussed here, *G. cantalica* would appear best placed in the genus *Gomphoneis*.

As indicated by both HUSTEDT (1935) and SCHMIDT (1899), the nature of the chambered areas suggests a relationship with members of the genus *Pinnularia*. In many *Pinnularia* species, the axial plate is expanded from the axial area, then terminates to expose interstriae internally. At a distance from the margin on the valve, another subtending lamina forms and extends from the valve face over the mantle (JACKSON, 1980; COX and ROSS, 1981; KRAMMER, 1982b). This results in chambers being formed between the valve surface and underlying laminae both from the axial area to the interstriae, and from the interstriae over the mantle. The area not underlain by the axial plate is delineated by two longitudinal lines on either side of the axial area formed by the edges of the

laminae. In *G. cantalica* the axial plate is well-developed, but the marginal lamina does not appear until almost the valve margin. This condition in *G. cantalica* produces the image of longitudinal lines on either side of the axial area positioned near midvalve. and a much-thickened valve margin,as depicted by SCHMIDT (1899). These observations do not correspond to HUSTEDT's (1935) illustration and description of two closely placed longitudinal lines positioned midway between the axial area and margin. The condition in *G. cantalica* differs from that described for *Gomphonema transylvanicum* Pant. which appears to possess longitudinal lines produced from underlying silica at the margin only (KRAMMER, 1982b).

ACKNOWLEDGEMENTS

We thank Dr. Charles W. REIMER, Curator of the Diatom Herbarium, Academy of Natural Sciences of Philadelphia, for tracking down and providing us with the Héribaud material. Research funding provided by grant BSR-8500263 of the National Science Foundation.

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OBSERVATIONS ON *AMPHORA* SPECIES (BACILLARIOPHYCEAE) IN THE BRITISH MUSEUM (NATURAL HISTORY)

IV. Some species from the subgenus *DIPLAMPHORA* Cleve

F.R. SCHOEMAN*, R.E.M. ARCHIBALD* and P.A. SIMS**

ABSTRACT. – Three *Amphora* species (*A. crassa* Gregory, *A. graeffeana* Hendey, *A. grevilleana* Gregory) belonging to the subgenus *Diplamphora* Cleve were observed on strewn slides in the British Museum (Natural History). Light microscope photographs of specifically marked specimens or examples corresponding to their descriptions have been included. Comments with regard to the authenticity of the materials examined and the suitability of the observed specimens as types for the species are made.

RÉSUMÉ. – Trois espèces d'*Amphora* (*A. crassa* Gregory, *A. graeffeana* Hendey, *A. grevilleana* Gregory) appartenant au sous-genre *Diplamphora* Cleve ont été observées sur des préparations microscopiques du British Museum (Natural History). Des microphotographies des spécimens identifiés en tant qu'espèces ou des descriptions sont jointes. Des commentaires sont faits concernant l'authenticité du matériel examiné et le bien fondé des spécimens observés en tant que types pour les espèces.

ZUSAMMENFASSUNG. – Drei *Amphora* -Arten (*A. crassa* Gregory, *A. graeffeana* Hendey, *A. grevilleana* Gregory) aus der Untergattung *Diplamphora* Cleve, wurden in Streupräparaten der Sammlungen des British Museum (Natural History) untersucht. Einbezogen sind Abbildungen von spezifisch markierten Individuen oder von solchen Exemplaren, die mit den Beschreibungen übereinstimmen. Ausserdem werden Kommentare zur Echtheit des geprüften Materials und zur Eignung der untersuchten Individuen als Typus-Exemplaren.

KEY WORDS : *Amphora*, Bacillariophyceae, light microscopy, type material.

INTRODUCTION

This is the fourth in a series of papers (SCHOEMAN & ARCHIBALD, 1985a, 1985b, 1985c) dealing with *Amphora* species of which the type material or type

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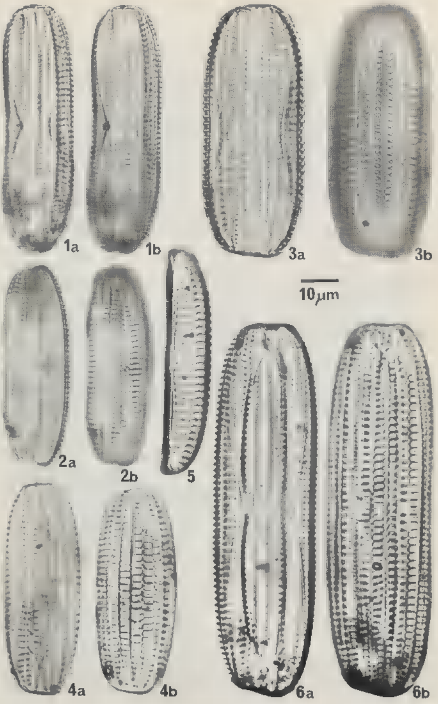

1a
1b
3a
3b
10µm
2a
2b
5
4a
4b
6a
6b

slides are to be found mainly in the British Museum (Natural History). These papers report on what an investigator may observe on a particular slide with reference to a specific species, and comment on the authenticity of the specimens examined.

In this paper only *Amphora crassa* Gregory has been recorded from southern Africa, occurring in the marine littoral of the southern and western coasts (GIFFEN, 1970, p. 266; 1975, p. 73; 1976, p. 381). The other two species were studied as a result of the complexities surrounding the taxonomy of *Amphora graeffii* Grunow (cf. SCHMIDT, 1874-1959, pl. 25, fig. 40), which is listed from Saldanha Bay on the west coast of South Africa (GIFFEN, 1976, p. 382, fig. 6). Since GIFFEN's illustration (op. cit.) introduced a suspicion that his identification was not quite accurate, we also examined *Amphora grevilleana* Gregory, which CLEVE (1895, p. 113) equated with *A. graeffii* as depicted by GRUNOW (*vide* SCHMIDT, *op. cit.*), and *Amphora graeffeana* Hendey, which is the new name given to CLEVE's concept of *A. graeffii*.

More detailed comments on these three taxa are made in the text, but these should not be regarded as the last word in their taxonomy.

MATERIALS

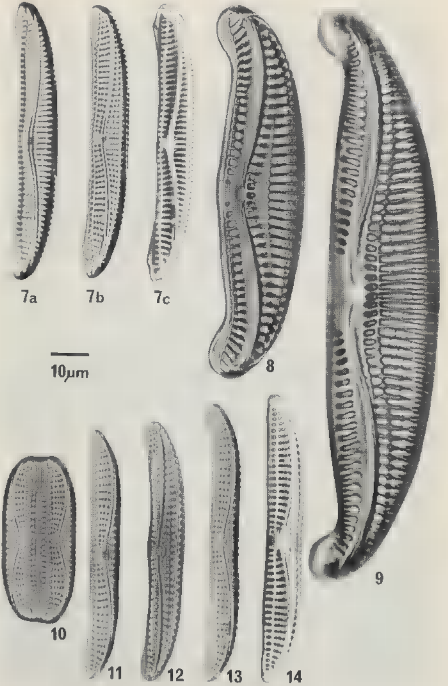
The diatom slides examined in this study are listed separately under the species dealt with. With the exception of the two HENDEY slides, all other slides are found in the collections of GREVILLE, PAYNE and TEMPERE & PERAGALLO (2nd Edition), housed in the British Museum (Natural History). The HENDEY slides (Nos. 6497, 6968) were obtained on loan from the HENDEY Collection (St Agnes, Cornwall) and examined in the British Museum (Nat. Hist.). The abbreviation BM, preceding a slide number, indicates a slide from the collections of the British Museum (Nat. Hist.).

OBSERVATIONS AND DISCUSSION

AMPHORA CRASSA GREGORY

GREGORY, 1857a, p. 72, pl. 1, fig. 35 (?).
GREGORY, 1857b, p. 524, pl. 14, figs. 94, 94b-d.

Plate 1. — Figs. 1-6 : *Amphora crassa* Gregory. Figs. 1a, b : BM 958. Glenshira. Ringed frustule (lectotype) at different levels of focus. Figs. 2a, b : BM 958. Glenshira. Another ringed frustule at two levels of focus. Figs. 2a - ventral view; Fig. 2b - dorsal view. Figs. 3a, b : BM 1341. Lamlash, Arran. Ringed frustule at two levels of focus. Fig. 3a - ventral view; Fig. 3b - dorsal view. Figs. 4a, b : BM 1193. Lamlash, Arran. Ringed frustule at different levels of focus. Fig. 4a - ventral view; Fig. 4b - dorsal view. Fig. 5 : [redacted] 1255. Arran. Ringed valve, showing valve face and dorsal mantle. Figs. 6a, b : BM 1192. Brodick Bay, Arran. Ringed frustule at different levels of focus. Fig. 6a - ventral view; Fig. 6b - dorsal view. — Fig. 1-6 : bright field illumination (B.F. Illum.).


7a
7b
7c
8
9
10μm
10
11
12
13
14

PERAGALLO & PERAGALLO 1897-1908, p. 208, pl. 46, fig. 5.
HENDEY 1964, p. 262.

Slides examined :

BM 955 «Glenshirra», Gregory C. 10. Coll. Greville. Ring No. 4.
BM 958 «Glenshirra», Gregory. Coll. Greville. Ring Nos. 1 (Figure 1), 2 and 3 (Figure 2).
BM 1192 Brodick Bay, Arran. Gregory 1857. Coll. Greville. Ring No. 3 (Figure 6).
BM 1193 Lamlash, Arran. Gregory 1857. Coll. Greville. Ring Nos. 1 (Figure 4) and 3.
BM 1196 Arran, Gregory 1857. Coll. Greville. Ring No. 6.
BM 1255 Arran, Gregory 1857. Coll. Greville Ring No. 3 (Figure 5).
BM 1341 Lamlash, Arran 1857, Gregory. Coll. Greville. Ring No. 8 (Figure 3).
BM 38612 Stomach of Holothurian, Alexandria. Coll. F.W. Payne (Figures 11-14).
BM 38615 Stomach of Holothurian, Alexandria. Coll. F.W. Payne. (Figure 10).
BM 38618 Porto Seguro, Brazil, Coll. F.W. Payne. (Figures 8, 9).
Hendey 6968 Bryher (Isles of Scilly). Coll. Hendey. EF M40 (Figure 7).

Notes :

This species was originally described by GREGORY (1857a) from the diatomaceous sand of Glenshira. In a subsequent paper (GREGORY, 1857b) he amplified the description and figured «the true *A. crassa*» from Lamlash Bay stating that the figure given in his original description (GREGORY, 1857a) «is not, at all events, the usual form. . . . . and it may possibly represent a different species». There are two slides of the Glenshira material, BM 995 and BM 958, bearing ringed examples of *A. crassa*, from which the lectotype slide can be chosen. The specimen on slide BM 995 (Ring No. 4) is a poor example, and therefore we have selected the second slide, BM 958, with three ringed frustules as the lectotype slide. The morphological details of these frustules (Figures 1, 2) correspond with GREGORY's (1857a, 1857b) two descriptions, but it seems evident that he did not observe clearly the structure of the transapical striae of the valves. These are coarsely punctate, and are not similar to the girdle striae. Despite this, we accept these examples as the true *A. crassa*.

We then examined two slides (BM 1341 and BM 1193) prepared from GREGORY's material collected in Lamlash Bay, where he found the species more frequently. A ringed frustule from each of these two slides is illustrated in Figures 3 and 4 respectively. Both examples correspond closely with GREGO-

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Plate 2. — Figs. 7a-c : *Amphora crassa* Gregory. Hendey 6968. Bryher, Isles of Scilly. Same valve under different forms of illumination and at various levels of focus. Figs. 8, 9 : «*Amphora crassa* Gregory» *sensu* F.W. Payne. BM 38618. Porto Seguro, Brazil. Figs. 10-14 : *Amphora crassa* Gregory var. *punctata* Grunow (= *A. crassa* Gregory). Fig. 10 : BM 38615. Stomach of Holothurian, Alexandria. Frustule, ventral view. Figs. 11-14 : BM 38612. Stomach of Holothurian, Alexandria. Various valves. — Figs. 7a, 8-13 : B.F. Illum. Fig. 7b : oblique bright field illumination (O.B.F. Illum). Fig. 7c, 14 : phase contrast illumination (P.C. Illum.).

RY's (1857b) later description, and are identical to the specimens on the lectotype slide.

Three additional slides from Arran, the same general environment in which lies Lamlash Bay, were also examined. Two of these (slides BM 1196, BM 1255) are not further qualified with a reference to a specific locality, while the third (BM 1192) comes from Brodick Bay. The specimen in Ring No. 6 on slide BM 1196 is poor and of little diagnostic use. On the other hand, the example on slide BM 1255 in Ring No. 3 (Figure 5) shows a valve in more or less the same aspect as that illustrated by GREGORY (1857b) in figure 94c of his plate 14. This example demonstrates a valve turned towards the dorsal mantle and illustrates more clearly a longitudinal line (costa ?) dividing the valve face from the dorsal mantle. Figure 6 depicts a much larger example from Brodick Bay (Slide BM 1192 Ring No. 3) having the same structural characteristics as the lectotype.

To obtain a more modern concept of *A. crassa*, a slide from the HENDEY collection (Hendey 6968) was borrowed by the senior author and examined in the British Museum. HENDEY personnally indicated the specimen (Figure 7) illustrated here, which has been marked with an England Finder (EF) co-ordinate as shown above. This example clearly shows the typical characteristics of *A. crassa*. The punctate nature of both the dorsal and ventral striae is very evident in this specimen (Figures 7a and 7b), while a phase contrast view (Figure 7c) demonstrates the dorsal longitudinal line as seen in Figure 5, but in a different plane of viewing. Another feature plainly seen in Figure 7c is a ventral longitudinal line interrupting the striae near the ventral margin. This ventral costa (?) has not been mentioned in the earlier descriptions (GREGORY, 1857b; PERAGALLO & PERAGALLO, 1897-1908) nor in HENDEY's (1964, p. 262) more recent circumscription. As this structure is also clearly visible in examples from Glenshira and Lamlash Bay on slides BM 958 (Figure 2a), and BM 1193 (Figure 4a) respectively it would appear to be a diagnostic character, which should be noted in future descriptions of the species.

The F.W. PAYNE collection of the British Museum (Natural History) contains a slide (BM 38618) from Porto Seguro in Brazil, on which the only identification given is *A. crassa*. A mere glance at the size, valve shape and striae structure of the specimens observed here (Figures 8, 9) is sufficient to show a case of complete misidentification. We have however, not been able to identify this taxon yet, but believe it to be akin to *Amphora egregia* Ehrenberg.

Finally, two further slides in the F.W. PAYNE collection are labelled as containing *A. crassa* var. *punctata* Grunow. A frustule on slide BM 38615 (Figure 10) and valves from slide BM 38612 (Figures 11-14) have been illustrated. Apart from the very distinctly punctate striae, there seems little to differentiate these examples from the lectotype (Figures 1, 2) or the other specimens in Figures 3-7. Figure 14 shows particularly clearly the interruption of the ventral striae by a ventral longitudinal line (costa ?) as described above in the HENDEY specimen (Figure 7c). We therefore support VANLANDINGHAM (1967, p. 207) in accepting the var. *punctata* as synonymous with *A. crassa*.

Dimensions of specimens examined :

Length 45.0-98.0 μm; breadth of frustule 17.5-28.0 μm; breadth of valve 8.0-12.0 μm; dorsal striae at the centre 6-8 in 10 μm, near the centre 5-8 in 10 μm and at the poles 6-9 in 10 μm; ventral striae near the centre 5-8 in 10 μm; striae on the girdle bands 5-7 in 10 μm.

AMPHORA GRAEFFEANA HENDEY

HENDEY, 1973, p. 317, figs 12-19.

Slides examined :

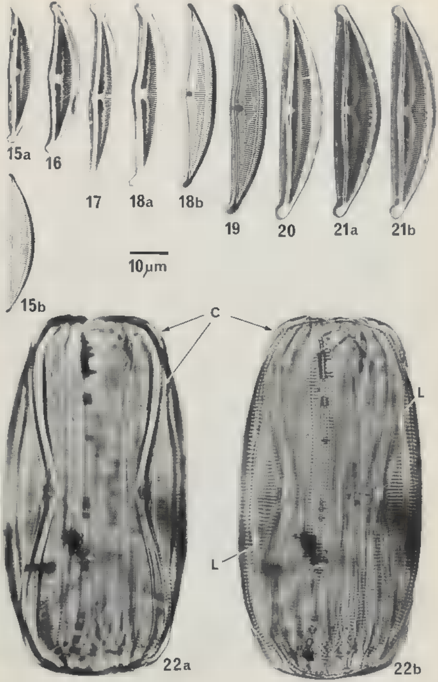
Hendey 6497 Porthleven. Cornwall. Coll. Hendey. (Figures 15-19).
BM 68466 Port Townsend Washington, U.S.A. Coll. Tempère & Peragallo (2nd Ed.) slide No. 120 (Figures 20, 21).

Notes :

Amphora graeffii Grunow (ex SCHMIDT,1874-1959, pl. 25, fig. 42) has been recorded from the marine littoral of South Africa at Saldanha Bay (GIFFEN. 1976 p. 382. fig. 6). There is some doubt about this identification. and the complications surrounding the true identity of *A. graeffii* makes it difficult to assess its accuracy. Although detailed taxonomic studies are beyond the scope of this series of papers, some background information in this case is required to explain why we examined the two slides mentioned above.

Amphora graeffii has no formal written diagnosis, but its identity is based on an 1875 drawing by GRUNOW in SCHMIDT's atlas (SCHMIDT, 1874-1959, pl. 25, fig. 42). CLEVE (1895, p. 113), however, considered this specimen to be *A. grevilleana* Gregory (see below), while he accepted GRUNOW's *A. graeffii* var. (*vide* SCHMIDT *op. cit.*, pl. 25, fig. 40) as the true *A. graeffii*. HENDEY (1973, p. 317) agreed with CLEVE. but felt it necessary to rename CLEVE's concept of *A. graeffii*, as it differed from the original GRUNOW drawing bearing that name. Consequently, having examined numerous specimens from Porthleven agreeing with CLEVE's description, HENDEY renamed it *Amphora graeffeana*. We have examined a number of examples from HENDEY's Porthleven slide (Hendey 6497). These specimens (Figures 15-19) agree very closely with GRUNOW's drawing of *A. graeffii* var. (SCHMIDT, 1874-1959, pl. 25, fig. 40; *vide* GRUNOW drawing collection, Naturhistorisches Museum, Vienna, Bilder-Sammlung No. 3030) in shape of valve and the distinct longitudinal line cutting across the dorsal transapical striae near the dorsal margin (Figures 15b, 18b, 19). The Porthleven examples differed from it only in the shape of the axial area, which is relatively wider and often becomes constricted to varying degrees at the centre of the valve by the lengthening of the central dorsal striae (Figures 17,18). It should also be noted that when ventral striae are visible, these are restricted to the valve apices (Figures 18b, 19).

PERAGALLO & PERAGALLO (1897-1908, p. 211, pl. 46, fig. 20; pl. 47, fig. 4) also appear to have accepted CLEVE's (1895) ideas, but illustrate two


15a
16
17
18a
18b
19
20
21a
21b
15b
10μm
C
L
L
22a
22b

different forms as their concept of the true *A. graeffii*. The first of these (pl. 46, fig. 20) is something different to the Porthleven examples, but the second form (pl. 47, fig. 4) is remarkably similar to HENDEY's specimens (Figures 15-19). At the same time PERAGALLO & PERAGALLO (1897-1908, p. 211, pl. 46, fig. 14, 15) described and illustrated a yet smaller form, *A. graeffii* var. *minor*. We therefore examined slide BM 68466 (= Tempère & Peragallo slide No. 120) observing several examples of this variety (Figures 20, 21). These appeared to be almost identical with HENDEY's specimens from Porthleven; the only difference being a row of irregularly spaced flecks along the dorsal margin. These flecks arise apparently from local interruptions of the dorsal mantle striae. In these specimens restriction of ventral striae to the valve apices is clearly evident (see figures 20, 21).

To add further to the confusion surrounding the identity of *A. graeffii*, HENDEY (1964, p. 263, pl. 37, fig. 8) depicts yet another form under the name *A. graeffii* var. *minor*. This specimen lacks the wide axial area characteristic of both the Porthleven examples (Figures 15-19) and the var. *minor* on slide BM 68466 (Figures 20, 21), and also has ventral striae interrupted only at the central nodule instead of being restricted to the apical region. The latter feature agrees, however, more closely with GRUNOW's drawing of *A. graeffii* var. (SCHMIDT, 1874-1959, pl. 25, fig. 40). In a later paper HENDEY (1970, p. 154, pl. 3, fig. 31) used the same specimen (cf. HENDEY, 1964, pl. 37, fig. 8) to illustrate «*Amphora graeffii* (Grunow) Cleve» (*sic*) from Kuwait, though he did remark that the Kuwaiti examples correspond more closely to the var. *minor*.

It is evident from the remarks above that further careful study is required to resolve the taxonomy of *A. graeffii* and the taxa closely associated with it. Whether CLEVE's (1895) description really relates to the Porthleven examples or to the form found along the Welsh coast (HENDEY, 1964) or in Kuwait (HENDEY, 1970) is a matter for more intensive investigation than can be given to it here.

Dimensions of specimens examined :

Hendey 6497 : Length 37.0-55.0 μm; breadth of valve 8.0-10.5 μm; dorsal striae near the centre 18 26 in 10 μm.

BM 68466 : Length 56.5-68.5 μm; breadth of valve 10.0-14.0 μm; dorsal striae at and near the centre 18-22 in 10 μm, and at the poles 19-22 in 10 μm; ventral striae near the poles only, 17-20 in 10 μm.

Plate 3. — Figs. 15-19: *Amphora graeffeana* Hendey. Hendey 6497. Porthleven, Cornwall. Figs. 15a, b : same valve, different illumination. Figs. 18a, b : same valve, different illumination. Figs. 20, 21 : *Amphora graeffii* Grunow var. *minor* Peragallo & Peragallo. BM 68466. Port Townsend U.S.A. Figs. 21a, b : same valve at different levels of focus. Figs. 22a, b : *Amphora grevilleana* Gregory. BM 960. Glenshira. Scotland. Ringed frustule at different levels of focus. Note conopeum (C) and longitudinal line (L). — Figs. 15a, 16-18a, 20-21b : P.C. Illum. Figs. 15b, 18b, 19, 22a, b : B.F. Illum.

AMPHORA GREVILLEANA GREGORY

GREGORY, 1857a, p. 73, pl. 1, fig. 36*.
GREGORY, 1857b, p. 522, pl. 13, fig. 89.
HENDEY, 1964, p. 263, pl. 38, fig. 6.

Slides examined :

BM 960 Glenshira, Gregory. Coll. Greville. Ring Nos. 1 (Figure 22), 2 (Figure 23), 3 (Figure 24), 4.

BM 68466 Tamatave, Madagascar. Coll. Tempère & Peragallo (2nd Ed.) slide no 100.

Notes :

A. grevilleana was originally described by GREGORY (1857a) from the diatomaceous sand deposit at Glenshira, Scotland. In a subsequent publication, however, he (GREGORY, 1857b) stated that the frustule illustrated in the original description actually belongs to another species, which he called *Amphora fasciata* Gregory. To correct this mistake, a new drawing by GREVILLE (cf. GREGORY, 1857b, pl. 13, fig. 89) was produced to illustrate the frustule of the true *A. grevilleana*. At the same time GREGORY (1857b, p. 522) expanded the description of the species.

We examined one slide prepared from the Glenshira diatomaceous sand (BM 960). This slide had five rings marked as containing *A. grevilleana*. Rings 1-4 encircled specimens that did agree with the description of *A. grevilleana*, but ring No. 7 contained a form which was dubious. We have therefore ignored the latter, and have illustrated three of the remaining ringed specimens (Figures 22-24). None of these are particularly good, but they do show reasonably clearly the characteristic features of the species. The specimen (Figure 22) in Ring No. 1 was the clearest, and we have therefore illustrated it at different depths of focus. The frustules are broadly oval to linear with broadly rounded, somewhat truncate apices. The girdle bands are clearly striate, and on the ventral side, at least, are separated from each other by a narrow structureless band (Figures 22b, c), which agrees with GREGORY's (1857a) description. The striae on the dorsal girdle bands consist either of a single row of pores, or a double row of pores arranged alternately or an intermediate arrangement where the pores from a single zigzagging line. The valve is broadly linear, with a convex dorsal margin and a more or less straight to slightly convex ventral margin. The poles are broad, somewhat rostrate and turned to the ventral side. The arcuate raphe branches lie in a relatively broad axial costa extended on the dorsal side into a conopeum (C) which can be seen reasonably well in Figure 22 and particularly at the poles, where it appears to be expanded somewhat. The central pores are weakly deflected to the dorsal side. The dorsal striae are interrupted by a longitudinal line (L: Figure 22b) running fairly close to the dorsal margin. Towards the centre of the valve this line expands slightly and arches flatly more to the dorsal side of the valve.

With reference to striae structure, both GREGORY's descriptions state that the striae are moniliform, implying a single row of pores. A similar impression

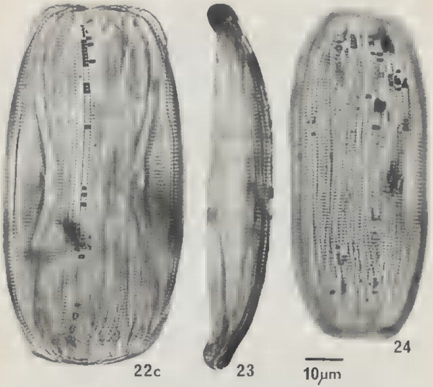


Plate 4. - Figs. 22c-24 : *Amphora grevilleana* Gregory. BM 960. Glenshira, Scotland. Ringed examples. Fig. 22c : same frustule as illustrated in Figures 22a and 22b, but at a different level of focus. Fig. 23 : single valve of a frustule (the complete frustule not shown here). Note raphe and dorsal longitudinal line. Fig. 24 : frustule in dorsal view, showing dorsal mantles and associated girdle bands. - Figs. 22c-24 : B.F. Illum.

is gained from CLEVE (1895, p. 113) and HENDEY (1964, p. 263), who describe the striae as being «coarsely punctate» and «punctate» respectively. On the other hand several illustrations of *A. grevilleana* in the literature (e. g. HENDEY, 1973, p. 317, fig. 20) depict a double row of alternating pores. In the specimens illustrated here in Figures 22 and 23 the striae structure is not very clear, but we gained the impression that they consist of a double row of pores arranged in quincunx. However, since the structure of the striae on the valve is sometimes very similar to the striae structure on the girdle bands (see above), it is possible that the valvar striae might also consist of a single row of pores.

Another point needing further clarification is the presence or absence of ventral striae. None of the descriptions or illustrations of *A. grevilleana*, that

we have studied mention the presence of ventral striae. However, in Figure 22c, striae with a structure similar to the dorsal striae are plainly evident on the ventral side of the valve. These fall short of the raphe leaving a hyaline band between the ventral striae and the raphe. This band appears to be at a slightly different level suggesting that it may be thickened or may represent a fold in the valve surface. In contrast, Figure 23 shows a specimen in which there is no indication of ventral striae, but this may be due to the angle at which the frustule is lying (Figure 23 represents only one valve of this frustule). On the other marked specimens on slide BM 960 (Ring Nos 3 and 4) ventral striae were also not visible. In this respect, HENDEY (1964, p. 263, pl. 38, fig. 6) illustrated an example having no visible ventral striae, which corresponds very closely to our Figure 23.

Finally, we examined one other slide on which the presence of *A. grevilleana* was indicated. This was slide BM 68466 (Tempère & Peragallo, 2nd Ed., slide No. 100) prepared from material collected at Tamatave, Madagascar. Despite being listed as present on this slide (TEMPERE & PERAGALLO,1907-15, p. 52) we found no examples on it.

Dimensions of specimens examined .

Length 85.0-104.5 μm; breadth of frustule 36.0-48.5 μm; striae near the centre 10-11 in 10 μm; striae on the girdle bands 12-13 in 10 μm.

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PHYSIOLOGICAL FEATURES OF SIX MICRO-ALGAE TO BE USED AS INDICATORS OF SEAWATER QUALITY

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and Marie-Claude BONIN[1]

ABSTRACT. — Reference is made to numerous physiological and behavioural characteristics of a few species of unicellular marine algae chosen as suitable tools for estimating sea water quality and for bioassay procedure : *Emiliania (= Coccolithus) huxleyi, Pavlova (= Monochrysis) lutheri, Dunaliella tertiolecta, Skeletonema costatum, Phaeodactylum tricornutum, Thalassiosira pseudonana* (clone 3H) and the neighbouring species *T. oceanica* (clone 13-1). Data taken from 450 papers are analyzed to give a comprehensive and comparative survey of cell size, growth rate, sinking rate, genetic variability, adaptation and tolerance to light intensity, light-dark cycle, temperature, salinity, organic and inorganic pollutants, heterotrophic potential, nitrogen, phosphorus, silicium and growth factors requirements in different ecological and cultural conditions.

Thoughts on the biochemical adaptations to changes in the environment are also included.

RÉSUMÉ. — A partir de l'examen de 450 travaux, les auteurs passent en revue les caractéristiques physiologiques et le comportement vis-à-vis des facteurs de l'environnement de six algues unicellulaires fréquemment utilisées dans les bioessais pour apprécier la qualité et la fertilité des eaux marines. Ce sont : *Emiliania (=Coccolithus) huxleyi, Pavlova (=Monochrysis) lutheri, Dunaliella tertiolecta, Skeletonema costatum, Phaeodactylum tricornutum, Thalassiosira pseudonana* (clone 3H) et l'espèce *T. oceanica* (clone 13-1).

Cette synthèse regroupe et compare entre elles des informations sur des caractères aussi différents que la taille des cellules, les taux de croissance, les vitesses de sédimentation, la tolérance et les mécanismes d'adaptation des algues aux variations de l'éclairement, de la température et de la salinité, la variabilité génétique, les besoins en éléments nutritifs (azote, phosphore et silice) et en facteurs de croissance, l'action de certains polluants minéraux et organiques.

KEY WORDS : Comparison between microalgae, sea water quality, *Dunaliella tertiolecta, Emiliana huxleyi, Pavlova lutheri, Phaeodactylum tricornutum, Skeletonema costatum, Thalassiosira pseudonana.*

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INTRODUCTION

In Oceanography, as in Limnology, a logical means of estimating the eutrophication level of a water mass and its ability to support algal biomass is to employ algal bioassays.

This method is already an old one, being attributed to ALLEN and NELSON (1910) and especially to SCHREIBER (1927), who described a technique for a «physiological analysis of seawater».

There are many ways to realize such experiments, depending both on the means and the aims of the research worker (see the general reviews in SKULBERG, 1978; MARVAN et al., 1979 and SHUBERT, 1984, among the more recently published). Modifications can be brought to the composition and to the relative concentrations of the nutrients added as enrichments, to the preliminary treatment of the inoculum, and to the way the test cultures are realized (enclosures, dialysis chambers, continuous cultures, etc., ELNABARAWY and WELTER, 1984; MAESTRINI et al., 1984a and 1984b; SCHELSKE, 1984, and WALSH and MERRILL, 1984 among others). Generally these methods have been applied more often for study of fresh than of seawater. As a result, standardization of the different methods is less far advanced in Oceanography than in Limnology.

That is particularly true of the choice of test strains, for, no synthetic study of this has yet been undertaken in Oceanography, in contrast to what has been achieved in Limnology (KOMAREK and LHOTSKY, 1979; KOMAREK and MARVAN 1979; WHITTON, 1984). The deficiency leads to the use of a large number of seawater strains in the tests (more than thirty, according to MAESTRINI et al., 1984b), and more often than not, the choice of strains is neither clearly justified nor submitted to preliminary research; moreover the physiological characteristics of some strains in use are totally unknown. Furthermore, some of these strains have been kept for a so short period in culture that they are not available for any further investigation. Criteria for the selection of the strains and conditions for their preservation are nevertheless of outstanding importance if we intend, on the basis of these biological tests, to give a relevant and exact interpretation of the ecological situations studied.

As KOMAREK and MARVAN (1979) and MAESTRINI et al. (1984a) maintained, the choice of the test algae should not depend only on the apparent ease of handling but also on their physiological, genetic and cultural characteristics. Balancing the relative advantages of these criteria should lead to the reasonable choice of a few superior strains upon which to base further studies. Standardization in the choice of the strains would contribute significantly to the standardization of methods and so allow a more realistic comparison between the potential fertility of various seawater samples.

HOW TO CHOOSE ALGAL TEST SPECIES

A good example of the way to establish that a species is suitable for fertility tests is afforded by the numerous papers on *Selenastrum capricornutum* which have led to the preferential use of that species in studies on polluted freshwaters. Since the initial work by SKULBERG (1964), there have been more than 200 papers concerning this species, with the result that its use in any further experiments is precisely defined (LEISCHMAN et al., 1979). From these data and the conclusions of KOMAREK and MARVAN (1979) and MAESTRINI et al. (1984a), it is possible to list the principal criteria that can be applied for the choice of marine strains as follows :

1) Wide geographical distribution : although it is not possible to find a strain able to grow in every ecological condition, the organism should have sufficient representation in the different oceans, at least at certain periods of the year. It is surprising to note that this point of view has largely been ignored by oceanographers, some of whom have not hesitated to use freshwater strains for testing seawater samples (review by MAESTRINI et al., 1984b). It is possible, and often advantageous, to use an endemic species well represented in the waters to be studied, and a simultaneous experiment with a second species of larger distribution is always interesting. The second species taken as a reference allows further comparisons between the data obtained and those of other authors concerning different ecological situations.

2) Well-known nutrient requirements : This requirement and the preceeding one conflict, since the nutrient requirements of free-living algae can vary greatly and moreover are for the most part unknown. The relative abundance of the algae varies considerably in time and space according to the nutritional status of the waters studied on account of the very extended range of physiological characteristics of the species. ROSEN (1981) achieved a synthetic study on the various taxa observed in Swedish lakes and on their abundance in relation to the physical and chemical properties of the different waters. To our knowledge, no such general review has been written for marine algae. It would be advisable to choose test algae differing greatly one from the other in their response so as to obtain good coverage of the nutritional qualities of the various seawaters, often poor and well-balanced in oceanic waters while rich and generally very unbalanced in the neritic zone. Now, such a choice can be validly established only if two kinds of information are gathered : first numerous studies on the quantitative and qualitative aspects of the distribution of the main species from areas of various hydrological characteristics are needed and then precise data on growth characteristics of each of those species under various culture conditions.

However, when experiments are undertaken to establish the nutritional requirements of an organism, one has to be very cautious about culture conditions, particularly physical factors (temperature, light level and salinity). This is especially so if the tests lead to comparison of growth rates or yields.

Moreover it is apparent from the literature that results can differ both with a single strain after different pre-treatment and, on the other hand, with different strains of the same species even after identical pre-treatment. Consequently a single experiment or study cannot be considered sufficient for evaluating physiological requirements. Since the burden of preliminary nutritional work, necessary to bioassay with untried algae, is so heavy that it is logical to choose strains that have been most often studied and for which maximum information is already available. Unfortunately, the species that have been well studied from the physiological point of view, have not always a wide distribution nor a major ecological role. They have been used in these various studies for several reasons : either they have been isolated accidentally and kept in culture collections where they were available, or they have been isolated with some particular aim very different from bioassay. On the other hand, when initiating an algal test series, it would be useful to choose several strains whose nutrient requirements are different, for example some auxotrophic organisms whose vitamin requirements are well defined and/or some strains whose strong affinity towards a particulate substrate is well known. Such a choice of several strains whose nutritional requirements are complementary allows a more complete appreciation of the nutritional capacity of the waters studied.

3) Good taxonomical characterization of the strains : Sexuality and other mechanisms involved in genetic transmission cause each individual within a same species to be potentially different from the others in its general and especially physiological characteristics. Every clonal culture has to be considered as an original. Therefore, origin, storage and routine cultural conditions have to be perfectly established for each strain used for tests. And when publishing, references of the strain used have to be given for each experiment. Unfortunately, ambiguities in origin and nomenclature of the clones are so frequent in the literature that valid comparisons between results are not always possible. To avoid confusion about the nature of the strain, it is recommended first to indicate the present-day taxonomical appelation but also to include any synonyms used for the strain in previous bioassay work.

4) Small genetic and phenotypic variability : Genetic evolution cannot be omitted within a clonal culture (NECAS, 1979); it is necessary therefore to keep the strain in such cultural conditions that change will be as small as possible, particularly during experiments as so to avoid hidden bias in the interpretation of the results.

For all these reasons, KOMAREK and MARVAN (1979) recommended that the strains be kept under their initial culture conditions until used in the tests. The growth rate of any genotype will normally remain constant in a particular environment once the strain is adapted to it. This steadiness however is not encountered in all algal families; for example the division rate of diatoms can vary over a period of several months even under constant environmental conditions (BRAND et al., 1981). Diatoms are in any case unsuitable for routine use in algal tests since these algae are diploid and homothallic and are liable to genetic recombination in «clonal» cultures, resulting in new genotypes among which

selection can occur (MURPHY, 1978; BRAND, 1981; GALLAGHER, 1982). From this point of view, dinoflagellates and coccolithophorids, which hardly reproduce sexually under laboratory conditions (*i. e.* saturated light intensity and nutrients in excess) would be more suitable as test organisms.

5) High growth rates : Rapid growth shortens the duration of the tests, especially when the measurement is biomass at the end of the exponential phase (algal growth potential = AGP). The shorter the experiment, the less the cost and the lower the risk of contamination by microorganisms and consequent change in the organic components of the water under study. Growth rates can vary considerably from one alga to an other (BAARS, 1981).

6) Ease of handling : The conditions for routine cultivation have to be sufficiently simple and the strain sufficiently resistant for the clonal culture to be kept easily and used without too many difficulties both in laboratory and at sea. Measurements of the parameters estimating growth or biomass have to be easy to record and reliable. The algae should easily be brought to homogenous suspension to allow ready methods of biomass measurement *e. g.* electronic counting. In that case cells should not be prone to settlement on the walls of the culture flasks, nor to show too large a variability in size or pigment composition according to nutrient status or culture conditions. Also they need to be unattached unicells.

CHOICE OF THE SPECIES

It is obvious that all these advantages cannot exist simultaneously in high degree in any one species. The choice of strains depends essentially on the aim of the study but the experimental constraints should not be neglected. The use of too many different strains by several workers makes it difficult or even impossible to compare results. Moreover, for a worker to use too many strains is to burden his own tests, since in this kind of experiment numerous measurements have to be taken at the same moment. It is better to analyze a great number of enrichments and to use fewer strains (MAESTRINI et al., 1984a).

For this reason, after having recorded all the information available on the physiology of the unicellular marine algae most often studied in culture and/or used in tests, and on the conditions of their use in bioassay, we have selected six most adequate according to the prerequisites from which the choice should be made. For various reasons, which will be detailed in each case, they appear to be the most suitable species for marine AGP studies.

They are : two haptophytes, *Emiliania (= Coccolithus) huxleyi* and *Pavlova (= Monochrysis) lutheri*, one chlorophyte, *Dunaliella tertiolecta*, and three diatoms, *Skeletonema costatum*, *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* (and the related species *T. oceanica*).

It is to be noted that these forms are also among those recommended by GUILLARD (1975) to feed the invertebrate juveniles in aquacultural systems.

Such species with a high synthesis potential are also recommended by industrial research institutes for developing biomass technologies (Anonymous, 1984).

EMILIANIA HUXLEYI (LOHMAN) HAY et MOHLER

Coccolithophorids are among the most widespread haptophytes (OKADA and McINTYRE, 1977). The life cycle of these algae involves two stages, a non-motile coccolithophorid stage and a motile flagellate stage, both reproducing their own kind by binary partition when kept separately in culture (PAASCHE, 1968; KLAVENESS and PAASCHE, 1979). Two species have been most widely used in cultures : *Cricosphaera (= Syracosphaera = Hymenomonas) carterae* (Braarud and Fagerland) Braarud, and *Emiliania (= Coccolithus) huxleyi* (Lohman) Hay and Mohler.

The second is a small cosmopolitan oceanic species (4 to 6 µm in diameter) particularly common and widespread in cool temperate waters. Its physiology is well documented, having been the subject of extensive studies on coccolith formation (PAASCHE, 1962, 1964; KLAVENESS and PAASCHE, 1971, 1979; KLAVENESS, 1972, 1976; SIKES and WILBUR, 1980, 1982; SIKES et al., 1980). Researches have been carried out on different calcified and motile or non-motile non-calcified strains. The motile stage only has uncalcified organic scales (KLAVENESS and PAASCHE, 1971). It is possible to induce a transition phase between flagellated cells and non-motile cells by growing the cultures in nitrogen-deficient media (WILBUR and WATABE, 1963). On the other hand, it is always possible to find varying percentages of non-calcified cells in cultures from a calcified strain (KLAVENESS, 1972); naked and also motile cells arise mainly in old cultures (SIKES and WILBUR, 1980). Resistance to salinity appears to be only moderate (lower than that of more euryhaline coastal species), the extremes of tolerance being 15 and 45 ‰ with an optimum between 19 and 32 ‰ (GUILLARD, 1963; PINTNER and PROVASOLI, 1963; BRAND, 1984). In certain areas, however, the species is most frequently found living at the lower limit of salinity and temperature (BIRKENES and BRAARUD, 1952; BERGE, 1962 *in* PAASCHE, 1968); and it has been observed that, when salinity is decreasing, growth is inhibited and the percentage of calcified cells increases. In these conditions, the increase in calcification is due to the greater tolerance of calcified cells to low salinity rather than to any restoration of calcification in non-calcified cells. A similar observation concerns the greater tolerance towards high pH in calcified than in non-calcified cells (SIKES and WILBUR, 1980, 1982). These cultural characteristics might even be used to maintain calcified cells in culture. In general, calcified and non-calcified cells have not the same growth behaviour : naked cells divide more rapidly than calcified cells (PAASCHE and KLAVENESS, 1970). Very different sinking rates were observed in well growing cultures for calcified ($1.3\ m.d^{-1}$) and for non-calcified cells ($0.28\ m.d^{-1}$) by EPPLEY et al. (1967). For this reason, the high sedimentation rate observed with coccolithophorids, compared to that observed

with many other unicellular algae according to SMAYDA and BIENFANG (1983) could make the bioassays difficult.

The conditions for coccolith formation are not clear. (1) BOROWITZKA (1977), and SIKES et al. (1980) considered that this formation increases the internal supply of CO_2 through a reaction involving HCO_3 influx. In fact, the rates of carbon fixation in *E. huxleyi* in culture, unlike many other algae, are C-limited at ambient levels of dissolved inorganic carbon in seawater (SIKES and WHEELER, 1982). The activity of the carbonic anhydrase is very low compared to that in other algae and does not allow *E. huxleyi* to achieve a high level of CO_2 in its cells and become carbon saturated. (2) Coccoliths may also help the absorption of light at very low intensities (BLANKLEY, 1971) and consequently growth under such conditions. According to EPPLEY et al. (1969) this makes it a formidable competitor for other phytoplankters at all nitrate concentrations. PAASCHE (1967) and BRAND and GUILLARD (1981) reported that growth of *E. huxleyi* is retarded by light periods shorter than 16 hours out of a total of 24 hours in light-dark cycles. BRAND and GUILLARD (1981) noticed that an oceanic strain of *E. huxleyi* can reproduce at all light intensities studied almost as rapidly in continuous light as in 14:10 light-dark regime. This response differs from that of the other species from oceanic areas, which are more or less harmed by continuous light. (3) According to SIKES and WILBUR (1982) the presence of coccoliths imparts a greater tolerance to low salinity, allowing calcified cells to grow when non-calcified cells do not. *Emiliania* as usually identified in nature bears coccoliths, which can be correlated with the fact that it is most frequently found living in low salinity waters.

BRAND and GUILLARD (1981) recorded that reproduction rates are generally lower in coccolithophorids than in diatoms but higher than in dinoflagellates. Like all phytoplankters, *E. huxleyi* shows a growth rate sensitive to temperature. WATABE and WILBUR (1966) recorded a division rate increasing from 0.2 - 0.6 div.d^{-1} at temperature near 7°C to 1.0 - 1.5 div.d^{-1} between 18 and 24°C. With another strain, PAASCHE (1967) found 0.1 - 0.6 div.d^{-1} at 12°C and 0.6 - 1.8 div.d^{-1} at 20°C. BRAND (1981, 1982a) observing the behaviour of 73 clones of *E. huxleyi* isolated from different parts of the western North-Atlantic, found reproduction rates ranging from 1.55 - 1.72 div. d^{-1} at 16°C to 2.03 - 2.45 div. d^{-1} at 26°C. This author obtained strong evidence for genetic differentiation of the species in the various areas of provenance. For example, the strains collected from the Gulf of Maine are better adapted to colder temperatures, reproducing more rapidly at 16°C and more slowly at 26°C, than a population from warmer waters as in the Sargasso Sea.

Several clones of *E. huxleyi* have been studied in experiments. Cultures were considered unsuitable, at least for the studies on coccolith formation, unless they contained a very high percentage of cells bearing coccoliths. Since cells in cultures tended to lose their ability to form coccoliths, it has frequently been necessary to reisolate from the wild and establish new clones (PAASCHE, 1964). Nevertheless two strains of *E. huxleyi* have been more extensively studied than others : strain «F-5», a naked form isolated by PAASCHE (1964) in 1959,

from the Oslo Fjord, and strain «BT-6», a calcified form isolated by Guillard in 1959 from the Sargasso Sea. Although the two strains look different and originate from waters of very different qualities, their nutritional characteristics are very similar, according to EPPLEY et al. (1969). These authors recorded K_S for NO_3-N and NH_4-N, ranging about 0.1 μg-at.l^{-1} for the two strains. The occurrence of this species in waters of very low nutrient content, such as the Sargasso Sea (HULBURT et al., 1960; HULBURT, 1982), or in very low illumination such as at the limit of the photic zone (MARSHALL, 1966, 1968), can be explained by its great affinity for nutrients coupled with its ability to make the best use of the available illumination.

This species, which is well adapted to take an advantage in the competition over other unicellular algae in oligotrophic waters, grows also in rich and dystrophic areas (BRAARUD, 1945, 1955; HULBURT and RODMAN, 1963). This cannot be accounted for by its adaptative nutritional characteristics but rather by its comparatively great resistance to pollutants and especially to heavy metals. as suggested by PINTNER and PROVASOLI (1963).

In other respects the heterotrophic capacity of *E. huxleyi* is not very large (PINTNER and PROVASOLI, 1963). Only urea can be utilized to any extent (ANTIA et al., 1975). Uric acid is not used (PINTNER and PROVASOLI, 1963) while amino acids are used very poorly (WHEELER et al., 1974). On the other hand, in common with many other algae, when mineral phosphorus is lacking, *E. huxleyi* can use phosphoric esters owing to an alkaline phosphatase, whose activity however is not optimal at seawater pH (KUENZLER and PERRAS, 1965).

The vitamin requirements are unusual for Chrysophyta since there is none for vitamin B_{12}, though there is one for thiamine.

These last physiological and cultural characteristics recommended *E. huxleyi* for certain bioassays (concerning for instance thiamine) and a combination with another species of developed heterotrophic abilities for the assay of waters poor in mineral nitrogen. In spite of a large variability observed in several clones, it is surprising, regarding the ease of handling, the presence of isolated cells and the rather high growth rates, that this microalga has not so far been used for fertility tests.

PAVLOVA LUTHERI (DROOP) GREEN

Pavlova (= Monochrysis) lutheri (Droop) Green, a small alga (5 to 7 μm) now classed in the Haptophyceae, is recorded from brackish supra-littoral rocky pools in Europe (DROOP, 1953, 1955b). The single clonal isolate, now much studied throughout the world, originated from the Isle of Cumbrae, Scotland in 1953 («SBBA strain 60» but known in many laboratories as «Mono L»). *P. lutheri* can occur in enormous numbers, more than 16.10^9 cells l^{-1} (DROOP, 1954a). It is extremely euryhaline and can be found in pools ranging from 0.1 to 60 ‰

salinity (DROOP, 1953, 1955b). The range tolerated in culture is equally great, the optimum appearing to be between 2 and 10 ‰ (DROOP, 1958a) or betbeen 5 and 35 ‰ (BRAND, 1984). CRAIGIE (1969) demonstrated that *P. lutheri* compensates the salinity of the medium by regulating its own internal osmotic pressure with new synthetized cyclitol 1,4/2,5 cyclohexanetetrol. The accumulation of cyclitol is directly proportional to the increase in osmotic pressure of the environmental medium. The synthesis of this component only takes place in the light.

P. lutheri was one of the first marine auxotrophs to be studied. DROOP (1954b) demonstrated a vitamin B_{12} requirement, with *Leichmanii* specificity (DROOP et al., 1959) and a thiamine requirement for the pyrimidine moiety (DROOP, 1958b). Bioassay for vitamin B_{12} with this organism (DROOP, 1955c) were among the first vitamin assays to be performed on sea water. They contributed to the growing idea that vitamins might account for some of the differences in fertility between different water masses (DROOP, 1957a, 1961a). Utilization of vitamin B_{12} by *P. lutheri* was the origin of the series of papers by DROOP (1968, 1973, 1974, 1975, 1977, 1983) and DROOP et al. (1982), in which the Cell Quota model of nutrient controlled growth was developed. The minimum requirement for vitamin B_{12} is of vanishingly low order, 3 molecules per μm^3 of algal protoplasm (DROOP, 1957b); the K_s for uptake of the free vitamin being of the order 0.12 pM and for growth 0.03 pM (DROOP, 1974).

Other aspects of nutrition are also fairly well known. Like most supra-littoral species, the affinity for nitrate and ammonium is low, the apparent K_s for uptake being of the order 0.5 μg-at. l^{-1} N (EPPLEY et al., 1969) and moreover dependent to some extent on growth rate (CAPERON and MEYER, 1972a, 1972b). *P. lutheri* can also use organic nitrogen but to a varying extent. Good growth has been reported on urea, uric acid, glucosamine but, with the exception of glycine, amino acids appear not to be available (DROOP, 1955b; ANTIA et al., 1975).

An alkaline phosphatase has been recorded, but low activity and a maximum at a pH well above that of sea water (KUENZLER and PERRAS, 1965). DROOP (1974, 1975) has shown that cells could store several times their limited cell quotas of phosphorus or vitamin B_{12} when these nutrients were supplied in excess while, according to TERRY (1980), only relatively small amounts of excess nitrogen could be stored as cell nitrogen when this was in excess. Furthermore, phosphate inhibits the uptake of nitrate even under N limitation (TERRY, 1982a). When phosphate is supplied in excess, intracellular P is accumulated as polyphosphates reaching as many as 70 % of the total P content of the cells (SAKSHAUG and HOLM-HANSEN, 1977). Both P (DROOP, 1975) and N (TETT et al., 1985) uptake are halted by depletion of vitamin B_{12}.

Some studies using *P. lutheri* have been carried out on the variation of the photosynthetic ratio according to the nature of the nitrogen source (NO_3 or NH_4) and to the extracellular O_2 and CO_2 concentrations (BURRIS, 1981). An analysis of the kinetics of fluorescence induction to estimate the potential

productivity of phytoplankton populations was undertaken by NEVEUX and JUPIN (1981).

P. lutheri, in common with other neritic species, is not very sensitive to pollutants. For example, with copper, growth is not inhibited by as much as $100 \mu g.l^{-1}$ (ERICKSON et al., 1970), and with fluoride, growth is 30 to 50 % inhibited by the very high concentration 150-200 $mg. l^{-1}$ (ANTIA and KLUT, 1981). VANDERMEULEN et al. (1983) carried out a very interesting study of the action of petroleum hydrocarbons on the behaviour of *P. lutheri*. They recorded that the motility of the alga is affected by concentrations of pollutant much lower than those acting on growth and that the response to a defined concentration of pullutant is independant of the exposure time. Similar observations have been made concerning other chemical pollutants, particularly heavy metals. This behaviour of *P. lutheri* at very low concentrations of pollutants is not of a cumulative nature since it is not time-dependent. It could therefore be used with advantage for toxicity studies in natural media.

The growth rate is rather low. DROOP (1974) recorded a maximum of 1.34 $div.d^{-1}$ at 19°C in continuous culture; SAKSHAUG and HOLM-HANSEN (1977) recorded values between 1.2 and 1.9 div. d^{-1} at the same temperature. GOLDMAN and CARPENTER (1974) found it to have the lowest growth rate of a dozen algae tested. A temperature dependence was observed by GOLDMAN (1979) (an increase of the specific growth rate (μ) from 0.9 to 1.2 d^{-1} between 15 and 23°C, *i.e.* from 1.3 to 1.8 div. d^{-1}).

One characteristic of *P. lutheri* allows easy handling in tests : its sinking rate, as shown by EPPLEY et al. (1967), is low (0.18 m. d^{-1}) for flagellates in exponentially growing cultures.

P. lutheri (as *«Monochrysis»*) is one of the algae very commonly used in aquaculture. Its advantages are (1) its good assimilation efficiency (DAVIS and GUILLARD, 1958; PERSOONE and CLAUS, 1980; UKELES, 1980; PEIRSON, 1983) and (2) its generally high level of proteins up to 53 % dry weight (TAUB, 1980). DAVIS and GUILLARD (1958) found this strain to give the higher yield when used as food for the american oyster *Crassostrea virginica*. DROOP and SCOTT (1978, 1982) and SCOTT (1980) developed models of the energy relations of *P. lutheri* and a rotifer predator in continuous culture.

This rather slow growing species with, however, well known physiology, might be useful for studying littoral and low salinity waters and wherever the vitamins B_{12} and thiamine are suspected of being limiting. Characteristics of this alga agree with some of the criteria given in the introduction and it is surprising to note that it has not been used more often in tests as indicator of seawater quality (MAESTRINI et al., 1984b). In particular, it could be profitably employed in fertility estimation prior to commercial exploitation.

DUNALIELLA TERTIOLECTA BUTCHER

Dunaliella tertiolecta Butcher (BUTCHER, 1952) is a small unicellular chlorophyte, 6 to 8 μm in diameter. It has been maintained in unialgal culture after

isolation from the Oslo Fjord by Føyn in 1928. The original strain «19/6a» is kept at Cambridge (England), but several other strains have since been isolated. One, designated «DUN», and often mentioned, appears to be similar to the preceeding though doubts still exist after several studies using it. Many studies have been carried out with other species of *Dunaliella*, especially *D. salina* (Dunal) Theodoresco (THEODORESCO, 1905), the type-taxon described by DUNAL in 1837.

During the last twenty years attention has been called to the genus *Dunaliella*, noticeable on many grounds (review by MASUK, 1972). The genus is well represented in fresh as well as in very saline waters (LERCHE, 1937). Indeed the only algae to develop in the Dead Sea belong to this genus (OREN, 1981; OREN and SHILO, 1982). Like all the other species of the genus and several chlorophyceae, *D. tertiolecta* is very euryhaline ranging from 3.5 to 120 ‰ (McLACHLAN, 1960). The mechanism of this adaptation has been studied by numerous authors (LATORELLA and VADAS, 1973; BEN-AMOTZ, 1975; BEN-AMOTZ and AVRON, 1978; JONES and GALLOWAY, 1979). They showed that the ability of the alga to develop in very saline waters depends on its ability to synthesize great quantities of glycerol. The special nature of the cell's surface, rich in cabohydrates («surface coat» (OLIVEIRA et al., 1980)) assists ionic exchange. ENHUBER and GIMMLER (1980) reported that significant amounts of glycerol continuously diffuse into the medium when there is a glycerol concentration gradient between the cells of *D. parva* and the medium.

The cell content of *Dunaliella* has been studied under various conditions of nitrogen and silicium nutrition (SHIFRIN and CHISHOLM, 1981). They noted that the lipid concentration remains low, even in case of strong nutrient deficiency, contrary to what is observed in most planktonic algae. LOEBLICH (1982) showed that the carotenoid concentration of *D. salina* can rise if salinity and light intensity increase, pH decreases and/or nitrogen and phosphorus become deficient. Accumulation of carotenoid affords photoprotection in higher light intensities. BOROWITZKA et al. (1984) using the carotenogenesis capacity of this alga succeed in the pilot-plant production of carotenoids in open basins.

D. tertiolecta can synthesize as much as 50 % its dry weight as protein. This is important and the reason why SPECTOROVA et al. (1981/1982) recommended the use of this alga for intensive production in fish farming. CUHEL et al. (1984) showed that protein synthesis occurs at night as well as during the day. Night synthesis uses carbohydrates and metabolic pool carbon elaborated during the day, which increases the protein production to food web and also modifies the nutritional value of phytoplankton to herbivores.

Division rates can vary considerably with light and temperature and the optimum conditions for growth are rather complex since they depend on a number of parameters. The temperature range for growth of *D. tertiolecta* is wide. Generally, growth is better towards the high end of the range. UKELES (1961) recorded a lower threshold of 9°C, while EPPLEY (1963) observed growth at 40°C with an optimum at 33°C. Between these limits, and with sufficient illumination, the division rate can reach the high figure of 4 or 5 div. d^{-1}

(EPPLEY, 1972). However, most authors give much lower rates (between 1.5 and 3 div. d^{-1}). Between 12.3 and 25°C (water temperatures frequently encountered by this alga) the rate is somewhat less, varying between 0.6 and 2.3 div. d^{-1} (EPPLEY and SLOAN, 1966). The growth characteristics of *Dunaliella* have been tested with cross-gradients of salinity, light intensity and temperature. For example, if salinity is reduced to 1/4 of that of seawater, the highest tolerated temperature is only 36°C and if salinity is increased to a value twice or four times that of seawater, this upper limit can be raised to 42°C (EPPLEY, 1963, *in* EPPLEY and STRICKLAND, 1968). Similarly, the optimal growth temperature increases with light intensity (JITTS et al., 1964). High salinities are better tolerated if light intensity and dissolved mineral carbon concentrations are higher (GINZBURG and GINZBURG, 1981).

It appears from several studies on carbon assimilation that this chlorophyte shows characteristics similar to those described by STEEMANN-NIELSEN and JØRGENSEN (1968) for *Chlorella*. Assimilation is preferentially achieved through the C_3 metabolic pathway and consequently depends on the activity of ribulose biphosphate carboxylase (Ribusco) for CO_2 fixation. But HCO_3 can also be assimilated, since a carbonic anhydrase has been found as in carboxylating algae.

In the surface waters, rich in nutrients, where the presence of an abundant phytoplankton leads to the increase of pH and consequently of the HCO_3 fraction, *D. tertiolecta* can still photosynthesize actively owing to its carbonic anhydrase. This ability gives an advantage, in salt marsh waters, when competing with other chlorophyceae lacking this enzyme (LOFTUS et al., 1979). But *Dunaliella* cannot remain dominant in very confined or insufficiently buffered media when the pH rises above 9.3 (GOLDMAN et al., 1982a; 1982b). In such conditions, diatoms such as *Phaeodactylum tricornutum*, whose C_4 or C_4-like metabolism can become very active due to the direct incorporation of bicarbonate, tend to dominate. Like numerous algae of the *Chlorella* type, *D. tertiolecta* shows an adaptation to decreasing illumination such that the number but not the size of the photosynthetic units increases. Consequently it cannot adapt rapidly to the variations of light intensity to which planktonic algae are submitted in the euphotic layer due to turbulence. This explains the abundance of *D. tertiolecta* in surface waters when not agitated. A relation between photosynthetic activity and nitrogen deficiency has been observed by TURPIN (1983). He found that the C-fixation of NH_4 - limited cells of his strain «NEPCC-1» drops when NH_4 is again supplied at a sufficient level. This decrease is of short duration, since the NH_4 supply soon leads to an acceleration of the photosynthetic processes. GOLDMAN et al (1981) had found that growth of the strain «Dun» was not affected under the same conditions (N deficiency followed by N supply). ELRIFI and TURPIN (1985) analyzing these conflicting results showed that the initial decrease of C- fixation varies mainly from one strain to the other.

Nitrogen assimilation by *D. tertiolecta* is also well-documented. Its affinity towards mineral nitrogen varies with the salt species. Thus, EPPLEY et al.

(1969) quote K_S for uptake of 0.1 μM for NH_4-N and 1.4 μM for NO_3-N at 18°C. But K_S can also vary with temperature : with *Dunaliella* sp., THOMAS and DODSON (1974) found 0.28 μM at 15°C and 0.95 μM at 25°C for NO_3-N. *D. tertiolecta* is resistant to very high NH_4 concentrations, up to several hundreds $\mu g\ l^{-1}$ in the culture medium (ANTIA et al., 1975; THOMAS et al., 1980a). PAASCHE (1971) found that when the concentrations were very high (600μM) growth was always more rapid on ammonium than on nitrate under all light intensities and light/dark cycles. But when the concentrations are low, in continuous light, growth rates depend solely on them, and are independent of the N species, whether NO_3, NH_4, NO_2 or urea (GOLDMAN and PEAVEY, 1979). In natural conditions, BIENFANG (1975) showed that, if nitrogen becomes limiting, the NO_3 assimilation is reduced but not suppressed by NH_4. In batch cultures, the suppression of nitrate assimilation following nutrient additions can be due to the disparity of the uptake velocities, that of NH_4 being greater than that of NO_3. DORTCH et al. (1982) came to the same conclusions as Bienfang in N-deficient conditions but added that in N-sufficient conditions uptake rates for NO_3 and NH_4 are rather similar. These authors explain the differences between NO_3 and NH_4 assimilation by differences in their uptake mechanisms. Therefore, previous work suggesting the inhibitory action of NH_4 on nitrate uptake might need to be re-evaluated. Transients in ammonium uptake have also been studied in *D. tertiolecta* by GOLDMAN et al. (1981) and GOLDMAN and GILBERT (1982). Generally, nitrogen uptake of an alga can be very rapid during very short period if the alga is momentaneously submitted to high NH_4 concentrations. This absorption is less rapid in *D. tertiolecta* than in other algae but can have a longer duration. On the other hand, uptake rates are rather similar whatever the growth rates are, which is not the case in diatoms. In N-poor oligotrophic waters, where main source of nitrogen is ammonium sporadically provided by animal excretion through micropatches, *D. tertiolecta* cannot take a selective advantage on other algae whose uptake is more rapid.

D. tertiolecta grows very well on urea (ANTIA et al., 1975; SPECTOROVA et al., 1981/1982), and urea can easily be supplied in aquacultural systems since it is an inexpensive product. By contrast, growth on amino acids is less efficient (WHEELER et al., 1974). For example, the considerable concentration of 25 mM has to be reached for growth to take place on glycine (BERLAND et al., 1979).

Organic phosphorus can be used as P-source, although *D. tertiolecta*, which shows an acid phosphatase activity (ANTIA and WATT, 1965), has no external phosphatase (KUENZLER, 1970). *D. tertiolecta* needs no additional growth factors (PROVASOLI, 1963).

The greater tolerance of this alga to environmental conditions is increased by its easy adaptation to pollutants, particularly heavy metals (MANDELLI, 1969; ERICKSON et al., 1970; OVERNELL, 1976; CHIAUDANI and VIGHI, 1978; FISHER et al., 1984). With copper, ERICKSON et al. (1970) and CHIAUDANI and VIGHI (1978) quote 3 mg l^{-1} as an upper limit for toxicity. Instead of the total concentration of Cu, HAWKINS and GRIFFITHS (1982) used ionic

activity, as defined by SUNDA (1975), *i.e.* *p*Cu which is the negative logarithm of Cu^{++} activity. These authors confirmed a strong resistance to copper : division rates remained constant even with *p*Cu under 8, that is an ion concentration 3 orders above that showing down growth of a dinoflagellate or of *P. tricornutum* (*p*Cu = 11).

GOTSIS (1982), working on the neighbouring species *D. minuta*, showed complex interrelations between some heavy metals when added simultaneously at the beginning of the exponential growth phase. In his experiment, selenium and mercury, and selenium and copper were coupled; each metal exhibited an antagonistic action on the other, thus leading to a decrease of the overall toxicity. The causes of this antagonistic effect are still unknown.

RIISGARD (1979) showed that very high concentrations of copper decrease the efficiency of ionic regulation when *D. marina* was submitted to osmotic shocks. In 1980, RIISGARD et al. suggested that the metal acted on the mechanisms involved in cell-volume regulation, directly related to the osmotic regulation. Since the speciation of heavy metals is very dependent on the physical chemistry of the medium (DROOP, 1961b), it is not surprising to see effects due to pH, as those found by PARRY and HAYWARD (1973) using zinc. SUBBA RAO (1981a) recorded additional information on the behaviour of *D. tertiolecta* in culture toward metals. To provide an optimal doubling rate, rather high concentrations of metal had to be supplied, equal to that of the F_1 medium of GUILLARD and RYTHER (1962), while for other algae such as the diatom *Skeletonema costatum* the optimal rate was reached with concentrations of metal hundred times lower than that of the F_1 medium. Even if the alga is tolerant toward heavy metals, it nevertheless accumulates them in its cells. This aptitude has been observed in the case of various metals by RILEY and ROTH (1971), and with cadmium by JENNINGS and RAINBOW (1979a) who noted that Cd can be accumulated in the cell up to 1350 times its initial concentration of *ca* 1 ppm. This phenomenon has dangerous consequences in the food chain since metals can be transferred and be accumulated tenfold by grazers (JENNINGS and RAINBOW, 1979b).

SUBBA RAO and PLATT (1982) found a toxic effect on the part of salicylate eserine, a chemical occasionally used as an inhibitor of zooplankton metabolism in experimental systems. The effect was however less strong on *D. tertiolecta* than on the other algae they studied. On the contrary, sensitivity of *D. tertiolecta* to pesticides is of a similar order to that observed in diatoms (WALSH, 1983). Another proof of the high resistance to abnormal environmental factors is its tolerance towards temperature shocks experienced by confined phytoplankton populations, for example in cooling systems of thermal power stations. SELLNER et al. (1982), cultivating *D. tertiolecta* at 20°C and submitting it to various temperature shocks, studied the modifications appearing in fluorescence with and without DCMU addition. They observed an ability to recover an F_b/F_a ratio similar to that of the control for shocks of + 5° and + 10°C and they showed that these still very rapid shocks do not inhibit the photosynthesis of the alga. However, *Dunaliella* does not easily survive cryopre-

servation. BEN-AMOTZ and GILBOA (1980a) found that ability to survive is improved (up to 27 % of the control) if they used a two-step cooling technique and added a protective agent to the culture medium.

All the properties we have noted here for *Dunaliella* are those of an alga typical of rather still neritic waters rich in nutrients. But *Dunaliella* also has other intrinsic qualities that make it a good tool for tests. Thus, in spite of possible variations in salinity (5 to 35 ‰), SPECHT and MILLER (1974) and CHIAUDANI and VIGHI (1978) found a linear relation between biomass and nutrient content of the waters. This property can be very useful in tests of AGP in which final biomass is measured after nutrient are exhausted. This alga is also very easy to handle in culture. Its sinking rate during active growth is very low, which is another proof of adaptation to scarcely agitated or even standing waters : EPPLEY et al. (1967) mentioned 0.39 m. d^{-1} and BIENFANG and HARRISON (1984) only 0.01 m d^{-1} under nutrient-replete conditions whereas they give a mean value of 0.15 m. d^{-1} for the nanoplankton species they studied. *D. tertiolecta* remains well in suspension and has no tendancy to become attached to the walls of the culture vessels. Sampling and counting errors can therefore be minimal (EPPLEY and COATSWORTH. 1966). The qualities of this alga recommended it particularly for fertility test with littoral and enclosed waters where there are likely to be large variations in temperature and salinity and the possibility of pollution. Furthermore, its resistance to external factors allows a qualitative and quantitative measurement of the nutrients brought with pollutants, especially the organic ones such as urea or organic phosphorus, with only slight perturbations of the tests due to heavy metals or salinity changes.

SKELETONEMA COSTATUM (GREVILLE) CLEVE

Skeletonema costatum (Greville) Cleve is a diatom of world-wide distribution in coastal waters. Intertropical records however lay perhaps be more properly ascribed to the very closely related *S. tropicum* (HULBURT and GUILLARD, 1968). In temperate regions it occurs typically in spring, but may persist through the summer to the autumn overturn. *S. costatum* blooms can be very heavy (TRAVERS, 1973), up to 25 x 10^6 cells. l^{-1} (DROOP, 1957b) or even more than 100 x 10^6 cells l^{-1} (KARENTZ and SMAYDA, 1984), and may represent more than 90 per cent of the phytoplankton biomass (SMAYDA, 1957; PRATT, 1959; KARENTZ and SMAYDA, 1984) or even the whole population (BELL and SAKSHAUG, 1980). *S. costatum* is a very small diatom. Its size range in nature varies from 3 to 22 µm (CUPP, 1943; GUILLARD et al., 1974). However, its two dimensions vary according to nutrient concentrations and cell enlargement processes. BIENFANG et al. (1982) found a width (apical axis) between 1.9 and 2.9 µm and a length between 6.8 and 11.4 µm relative to N, P or Si replete or deplete cells.

S. costatum is not easy to maintain in culture, especially in bacteria-free conditions in defined media. Cultures tend to die quickly once the stationary

phase is reached (DROOP, 1962) and the vigour of cultures and the sensitivity to physical and chemical factors is to a great extend dependent upon the state of the population *vis-à-vis* the sexual (auxospore) cycle. Great variability can be observed both morphological and physiological, in parallel sub-cultures from the same clone (CASTELLVI, 1971; BERLAND et al., 1973). DROOP (1961b, 1962) has drawn attention to the importance in this connection to the state of reduction of iron in artificial media.

A reason why *S. costatum* is so ubiquitous within the neritic area is that it is able to adapt easily to temperature and salinity changes. Data in the literature and those collected by KARENTZ and SMAYDA (1984) indicate temperature tolerance limits of 0° and 30°C and division rates, which can become very high, varying with temperature. Thus, CURL and McLEOD (1961) quoted 4.28 div. d^{-1} at 20°C, SAKSHAUG and HOLM-HANSEN (1977) from 2.9 to 3.5 div. d^{-1} at 19°C. YODER (1979a) and HULBURT (1982) quoted a maximum division rate of 3.27 and 2.21 div. d^{-1} at 25°C respectively. In large enclosures exposed in a Norwegian fjord and under natural conditions BROCKMANN et al. (1977) found 2.1 to 2.4 div. d^{-1} at temperatures ranging from 15.4 to 17.3°C and EBERLEIN et al. (1983) 0.6 div. d^{-1} at very low temperatures (1 to 3°C). More generally division rate increases predominantly between 0 and 10°C and less rapidly for higher temperatures (JØRGENSEN, 1968). Various values of Q_{10} for growth rate have been recorded : Q_{10} = 3 between 5 and 10°C, 1.7 between 10 and 20°C and between 20 and 30°C (SMAYDA, 1973); Q_{10} = 2.25 between 2 and 24°C (FALKOWSKI, 1977) and Q_{10} = 1.8 in natural conditions (HEGSETH and SAKSHAUG, 1983). Anyhow, this increasing is not linearly related to temperature. And YODER (1979a) personally concluded that the daily division rate is exponentially related to temperature between 0 and 10°C but not above this limit. The differences between the values indicated by the different authors for growth rate can originate in the experimental conditions, more precisely light and nutrient conditions, but also in the nature and origin of the strains used. GUILLARD et al. (1974) cultivating five clones found only slight differences between them, which led these authors to give a mean value of 0.25 div. d^{-1} at 0°C, 2.25 at 28°C; there was no growth above 31°C. On the contrary, FURNAS (1982b) clearly demonstrated that variability is very large among the individuals from a natural population in a given water. This author studied 49 samples taken off the Narragansett Bay waters during summer blooms when temperature of the water varies between 16.1 and 22.3°C and then cultivated in growth chambers. He found that in 4 % of the samples *S. costatum* died, in 4 % the growth rate for *S. costatum* was 0.5 - 1 div. d^{-1}, in 18 % it was 1.01 - 2, in 33 % it was 2.01 - 3, in 18 % it was 3.01 - 4 and in 22 % it was higher than 4 with a maximum value of 5.9 div. d^{-1}; however most of the authors working in this area found values often lower than 2 div. d^{-1} (VARGO, 1976; YODER, 1979a; HITCHCOCK, 1980). Division rates higher than 3 are scarce in spite of the 5 div. d^{-1} at 24°C recorded by FALKOWSKI (1977). FURNAS (1982a) thinks that these values are different from one author to the other because of the various experimental methods used. Some methods, like those

using dialysis chambers, tend to underestimate the real division rates of algae in their natural conditions. However that may be, the high growth rates of *S. costatum* give it an advantage (even at low nutrient concentration (FURNAS, 1982b)) over other species especially flagellates. Several studies have indicated that *S. costatum* prevails more easily over its competitors at temperatures close to or lower than 10°C. GOLDMAN and RYTHER (1976) and DE PAUW et al. (1980) observed that this diatom can grow fast in enriched outdoor tanks and was thus a potentially interesting food source for aquaculture systems. *S. costatum* can however also be dominant among planktonic populations at higher temperatures : 25°C (GOLDMAN and RYTHER, 1976), between 15 and 20°C (LELONG et al., 1980), 21°C (RHODHOUSE et al., 1983). The good adaptation to low temperature is shown by the remarkably constant adenylate ratios or energy charges as defined by ATKINSON and WALTON (1967) : FALKOWSKI (1977) thus found adenylate ratios almost equal between 2 and 30°C and HEGSETH and SAKSHAUG (1983) cultivating *S. costatum* in dialysis chambers in a Norwegian fjord showed its ATP concentration to be independent of the temperature of the environmental medium.

Most authors, YODER (1979a) excepted, noted a decrease in cell volume following an increase in temperature. For JØRGENSEN (1968) the mean volume is 218 μm^3 at 7°C but only 127 μm^3 at 20°C. Decrease in size goes with relative increase in carbon and nitrogen content by cell volume unit (GOLDMAN and RYTHER, 1976).

As with most photosynthetic unicells it is not possible to dissociate the effects of light from those of temperature. *S. costatum* is among the most frequently studied of the diatoms from this point of view (CURL and McLEOD, 1961; JITTS et al., 1964; STEEMANN-NIELSEN and JØRGENSEN, 1968; HITCHCOCK, 1980). Not only is the growth rate directly a function of these two factors but the lower temperature limit is a function of both light intensity and day length (JITTS et al., 1964). These authors observed an increase in mean size at a given temperature with increase in light intensity. The influence of these factors on cell size in centric diatoms is due to their influence on the timing and frequency of cell enlargement which requires a critical combination of temperature and light for its completion (HOLMES, 1966). MIGITA (1967) confirmed that in *S. costatum* too low a light intensity prevents the formation of zygotes, which can explain why the mean size diminishes under these conditions. Climatic differences might therefore be expected to influence diatom mean sizes, as suggested by WIMPENNY (1936). Large variations can be observed even in cultures maintained in constant conditions. RYTHER and GUILLARD (1962b) recorded a range of 3 to 15 μm. In similar nutrient conditions, division rates seem constant even for cells of various size. PAASCHE (1973a) noted this fact in cells whose axes vary between 3 and 6 μm. Variations in cell size are accompanied by variations in some cell constituents. In any case culture conditions influence this phenomenon. For example, PRAKASH et al (1973a) recorded a larger mean cell size as well as a higher chlorophyll *a* content in cells cultivated in dialysis systems than in cells from batch systems. But in both cases,

the maximum cell size occurs during the stationary phase. PAASCHE (1973c) noted a decrease in the chlorophyll *a* and protein to silica ratio with increase in cell size. Chlorophyll *a* however varies with nutritional status. When cells become nutrient deficient, variations in their chlorophyll *a* content increase and have to be added to those due to light-dark periodicity of the nycthemeral cycle (OWENS et al., 1980). Indeed the tenfold range in the chlorophyll *a* : carbon ratio observed during ageing of *S. costatum* cells is among the greatest known (STEELE and BAIRD, 1962; HOLME, 1966), for which reason chlorophyll *a* is not a suitable measure of biomass in assays with this species. For its growth *S. costatum* does not need very high light intensities, as shown by HOBSON and GUEST (1983) who calculated a daily compensation irradiation (Σ I comp.) between 1.8 and 11 J. cm^{-2} . d^{-1} or between 0.97 and 5.93 μE. m^{-2} . s^{-1} according to irradiance conversion factors from HARRIS (1978), which is low compared to other diatoms, and by COSPER (1982c) who found a maximum net growth efficiency for 130 μE . m^{-2} . s^{-1} illumination only. CLOERN (1978), and CLOERN and CHENG (1981) collected data from the literature concerning the photosynthetic capabilities of *S. costatum* and tried to find the areas where climatic and hydrological conditions in the San Francisco Bay were the closest to those experimental data. They concluded that the alga can develop intensively in shoal areas and thence spread out to the other parts of the basin.

According to MYKLESTAD et al. (1982) the ability to grow at low intensity can also be explained by the existence of an exo-glucanase in the cells of *S. costatum*. This enzyme would bring energy supply by providing glucose from the cell's own polysaccharides.

Another characteristic of *S. costatum* is the small difference in the number of divisions between light and dark periods within a nycthemeral cycle. Thus, COSPER (1982b), using constant light intensity during the light period, found that the greatest differences occurred between 130 and 650 μE . m^{-2} . s^{-1}, which seems to be intermediate and rather high values, and that divisions were more frequent during the second half of the light period. This phenomenon has been already observed by JØRGENSEN (1966) and UNO (1971) and has been also found in other diatoms by NELSON and BRAND (1979). However, when light intensity is very low or very high, the differences in the number of divisions become smaller. COSPER (1982a; 1982b) noted that these differences were reduced if there were large fluctuations of the light intensity throughout the light period and that losses through excretion and photorespiration led to a decrease of the net growth efficiency. On the other hand, EPPLEY et al. (1971) observed that during dark period *S. costatum* divided more frequently if it was nutrient depleted than if it was in normal nutritional conditions. The small difference in division rates is of great advantage when the biomass estimation is based on cell number counting.

From the same point of view, variations of turbidity during the diurnal cycle are related positively to carbon content whatever the cell number. ØSTGAARD and JENSEN (1982) found that empty frustules have only about 1 % of the turbidity of the equivalent living cells, a useful characteristic when turbi-

dity is used for evaluating growth. In this respect *S. costatum* is a very good test alga.

The wide salinity tolerance range of *S. costatum* is universally acknowledged (CURL and McLEOD, 1961; NAKANISHI and MONSI, 1965) from 3.5 ‰ the year round in the Baltic (LEVANDER, 1947) to 38 ‰ in the Mediterranean. But the results differ with the authors since QASIM et al. (1972) found more than 30 % of the phytoplankton crop belonging to the genus *Skeletonema* in coastal waters of India with a salinity close to 0 ‰ and RAVAIL and ROBERT (1985) mention that a strain isolated from estuarine waters cannot grow below 8 ‰. PAASCHE (1975) found no growth at 2 ‰, half maximal at 4 ‰ and maximum from 10 to 40 ‰. He also found no difference between cells isolated from neritic waters with salinities of 6 and 20 ‰. In contrast, BRAND (1984) reports than an oceanic strain isolated from the Peru upwelling region does not develop at a salinity of 5 ‰ whereas a neritic strain shows division rates ranging from 0.55 to 0.89 div. d^{-1} at this salinity. Adaptation is very rapid. There is some evidence that, in contrast to growth rate, the rate of photosynthesis is maximal at salinity below 20 ‰. Contrary to other species of the genus such as *S. subsalsum* from brackish waters (PAASCHE et al., 1975), *S. costatum* cells do not show any deformation even when growing in low salinity.

The auxospore cycle, in addition to its effect on cell size, affects the vigour of the population, post-auxospore cells being more vigourous than pre-auxospore cells. DAVIS et al. (1973) observed rates of division varying between 0 and 2.88 div. d^{-1} in cultures in which synchronous zygote formation had been induced. In natural populations also variations in metabolism have been ascribed to the auxospore cycle (PATTEN and VAN DYNE, 1968). Marked differences can therefore be expected in the response to adverse conditions, such as nutrient depletion, according to the status of a cultured population *vis-à-vis* the sexual cycle. More or less synchronization of sexuality brought about by replenishment of severely nutrient-depleted cultures may result in unpredictable delays in the response to enrichment and variation in growth rate from one culture to the next according to the degree of synchronization. A further complication noted by HARRISON et al. (1976), and very similar to that previously reported for *Pavlova lutheri* by DROOP (1974), is the so called «shift up» in kinetic parameters when conditions are favourable for fast growth. Parameter values (*e. g.* K_s values for uptake as those below) quoted without reference to this phenomenon loose much of their meaning.

The nutritional requirements of *S. costatum* are fairly well-known, but as one might expect from the previous paragraphs, reports can differ markedly one from the other. Much information has come from more general studies of nutrient kinetics (DROOP, 1970; EPPLEY et al., 1971; FALKOWSKI, 1975; FALKOWSKI and STONE, 1975; CONWAY and HARRISON, 1977; COLLOS and SLAWYK, 1979; DE MANCHE et al., 1979; TURPIN and HARRISON, 1979; DORTCH, 1982; MASKE, 1982; QUARMBY et al., 1982; DORTCH and CONWAY, 1984, among others).

The nitrogen uptake kinetics suggest *S. costatum* as a species well adapted to the neritic habitat. For example EPPLEY et al. (1969) reported a K_s of

0.5μg-at. l^{-1} NO_3-N and of 0.8 to 3.6μg-at. l^{-1} NH_4-N but these constants however varied with temperature : K_S far below 0.5μg-at. l^{-1} NO_3-N at 8°C and 1.0μg-at. l^{-1} at 28°C. COLLOS (1982a) gave a K_S near 3.1 μg-at. l^{-1} NO_3-N at 20°C, much higher than that of Eppley et al. ROMEO and FISHER (1982) found a K_S of 2.10 at 17°C but the differences between these authors are probably due to the fact they did not use the same strain. According to EPPLEY et al. (1969) the ability of *S. costatum* to grow in neritic winter or spring waters, which are poorly illuminated and often rich in nitrogen salts, and the abundance of this organism at the beginning of phytoplankton blooms can be explained by these high values. *S. costatum* can grow in ammonium-rich waters (up to 200μg-at. l^{-1}) after THOMAS et al. (1980/1981), which explains its abundance in shelf waters eutrophicated by sewage input, such as those of the Black Sea (MIHNEA and VOINESCU, 1978; MIHNEA, 1980) provided that this «positive pollution» bringing nutrients is not accompanied by a «negative pollution» carrying heavy metals or other toxics (WALSH et al., 1982).

Recently DORTCH (1982) and DORTCH and CONWAY (1984) attempted to explain some of the mechanisms governing the absorption of nitrogen salts and their incorporation into amino-acids. Dortch demonstrated that incorporation of ammonium into amino acids and protein synthesis are two processes occurring at different speeds. As a result, intracellular nitrogen tends to increase when cells previously nitrogen-depleted are transferred to a nitrogen-rich medium. This increase can be high. Thus, COLLOS (1982b) indicated that intracellular nitrate can reach 15 % of the total intracellular nitrogen if nutrition is perturbated, whereas it does not rise above 1 to 2 % when growth takes place in steady-state conditions. The slow rate of reduction from nitrate to ammonium explains why *S. costatum* excretes large amounts of nitrite when growing on nitrate. This excretion can be a quarter of the nitrate uptake during light period (SERRA et al., 1978a, 1978b; COLLOS, 1982a) and can be even higher in the dark (COLLOS, 1982a). It is also with *S. costatum* that DORTCH and CONWAY (1984) have demonstrated that simultaneous nitrate and ammonium utilization is more frequent than one concludes from the works of DUGDALE (1976), COLLOS and SLAWYK (1980) and SYRETT (1981) among others. DORTCH and CONWAY (1984) having verified that algae prefer ammonium to nitrate, added that ammonium-inhibition of nitrate uptake is not only controlled by internal ammonium and total amino acids as previously believed, but also depends on both the preconditioning of the cells, whether cultivated on nitrate and/or ammonium and the respective concentrations of the two compounds in the medium at the time of observation.

S. costatum can also grow on urea. McCARTHY (1972) mentioned a K_S of 1.41 μg-at. l^{-1} urea-N. Urea acts as ammonium in being preferred to nitrate or nitrite. The rate of urea uptake is constant whatever the nutritional status : whether in the presence of high concentrations of nitrate and nitrite, or predepleted *i.e.* nitrogen almost consumed or completely depleted, *i.e.* several hours after total nitrogen consumption (HORRIGAN and McCARTHY, 1981). This property also applies to ammonium, but to a lesser extent (HORRIGAN and

McCARTHY, 1982). The rapid urea uptake rate, even when the cells are not depleted, is very important in the species nutritive strategy in nature where various or even low urea concentrations are encountered.

Uric acid is also used, but to a lesser extent (GUILLARD, 1963). Data in the literature on amino acids are contradictory : WHEELER et al. (1974) found no growth on eight out of the nine amino acids they studied. Only glycine was used at a concentration of 200 μg-at. l^{-1}. BERLAND et al. (1979) however improved glycine utilization by increasing its concentration. On the other hand, FISHER and COWDELL (1982) observed growth on alanine, aspartic acid, glutamic acid, serine, threonine and valine at a concentration of 100 μg-at. l^{-1}. Fisher and Cowdell's results however cannot properly be compared to those of the other authors because they did not use the same strains and their algae were not not bacteria-free, so that amino acid utilization by *S. costatum* could be an artefact of bacterial break down of amino acids to ammonium. In any case if amino acids are used, it is only at concentrations far above the natural levels.

The phosphorus requirement is not precisely known, except that P-depleted cells contain an alkaline phosphatase (ANTIA and WATT, 1965) produced in rather small quantity, but maximal at pH 8.6 (KUENZLER and PERRAS, 1965). This phosphatase activity is lower than that of *Chaetoceros affinis* for example (MØLLER et al., 1975) but this does not imply that *S. costatum* can hardly use phosphate esters when mineral phosphorus is in shorter supply than nitrogen. Indeed, MYKLESTAD and SAKSHAUG (1983) demonstrate this activity in a natural population from the Trondheim fjord, where *S. costatum* can reach 99 % of the total phytoplankton during blooms. In these coastal waters that are influenced by unbalanced fresh waters the N/P ratio can vary considerably and populations can become phosphorus depleted. Phosphatase activity is the highest at cellular N/P ratio over 14, the N/P balance point for *S. costatum* being 12 as indicated by MYKLESTAD (1977). When orthophosphates are missing from the medium, *S. costatum* can use organic phosphorus, which counterbalances a certain disadvantage *vis-à-vis* other species. As a matter of fact, *S. costatum* can only stock a limited quantity of phosphorus as polyphosphate, up to 10 % of the total cell phosphorus even if the culture medium is overloaded with phosphorus. This ratio is far lower than that of *Pavlova lutheri* for example (SAKSHAUG and HOLM-HANSEN, 1977).

The silicon requirement of *S. costatum* is satisfied by very low levels. The K_s for uptake lies between 0.71 and 1.11 μg-at. l^{-1} Si, while maximum uptake (U_{max}) occurs at a concentration of *ca* 10 μg-at. l^{-1} Si (PAASCHE, 1973c), which is below that normally found in sea water. Since silicon uptake is largely dependent on conditions of illumination (DAVIS, 1976), it would not appear that this element often limits growth of *S. costatum* under natural conditions. However, JAHNKE et al. (1983) named silicon as a limiting factor for surface waters of a Norwegian fjord in an experimental enclosure, but only when Si slowed down under 1 μg-at. l^{-1} Si. PAASCHE (1973c) also reported that at 20°C and in saturating irradiance the K_s of *S. costatum* was among the lowest of the diatoms observed, which suggests that this species might be at an advan-

tage, especially in neritic regions when other nutrients are not limiting. The fact that frustule abnormalities characteristic of silicon depletion in cultures are seldom observed in wild populations also argues for Si limitation to be a rare occurrence in nature. The silicon content is independent of irradiation but varies with temperature. HARRISON et al. (1977a) found a large decrease of Si concentrations in summer in natural waters. PAASCHE's (1980) observation of a doubling of the Si/C ratio between 8 and 23°C is not contradictory, since in summer Si often decreases in natural waters, counterbalancing the effect of temperature.

BIENFANG et al. (1982) studied the effect of nutrient (N, P and Si) depletion on sinking rates of *S. costatum*. They found that the buoyancy responses to Si depletion was the most pronounced of the nutrient regimes examined, but the difference between repleted and depleted cells are rather small compared with those of other diatoms : about 0.25 to 0.5 m. d^{-1} for *S. costatum* and 0.25 to 2.25 m. d^{-1} for *Coscinodiscus wailesii* for example. For *S. costatum* these values are close to those given by EPPLEY et al. (1967) in growing cultures (0.33 m. d^{-1}), but strikingly higher than the value (0.04 m. d^{-1}) recently mentioned by BIENFANG and HARRISON (1984). *S. costatum* rate is one of the smallest observed among diatoms, which can be related to its small cell size.

All *S. costatum* isolates examined have been found to have a vitamin B_{12} requirement (*e. g.* DROOP, 1955a; GUILLARD, 1968) but none for thiamine (DROOP, 1958b) nor biotin (PROVASOLI, 1958), which latter two however may be excreted by cells (CARLUCCI and BOWES, 1970a). The vitamin B_{12} requirement is of the unispecific, *i.e.* «coli» type (DROOP, 1955a; 1957b; DROOP et al., 1959; GUILLARD, 1968). The requirement is of a very low order; DROOP (1970) estimated a growth K_s of 0.2 pM free vitamin. It has been demonstrated *in vitro* that inhibitors of *S. costatum* growth acting by B_{12} limitation are produced by competitors such as *Gonyaulax tamarensis* and *Cyclotella cryptica* at cell densities about those of nutrient-rich coastal waters (MESSINA and BAKER, 1982). Interactions of that type between species have to be taken into account when interpreting population successions in which *S. costatum* is involved. These natural binders can interfere with the toxics from industrial origin found in coastal waters and acting on growth, modifying the results of the tests.

Skeletonema costatum is sensitive to the action of heavy metals (OVERNELL, 1976; MIHNEA et al., 1980), but the response is very variable. The first effect of copper toxicity for example is a lengthening of the lag phase. The threshold for this according to BERLAND et al. (1977) is 200 $\mu g. l^{-1}$ and according to JENSEN et al. (1976) 10$\mu g. l^{-1}$. In general, differences in response may be due to strain differences (BRAEK et al., 1980; GAVIS et al., 1981). Thus, the *S. costatum* strains sampled from estuarine waters have a better tolerance towards Zn than those from oceanic waters (FISHER, 1981). These differences in the results may equally well depend on the culture medium or the culture regime. For example, heavy metal toxicity is very dependent upon the ionic balance and degree of chelation (DROOP, 1961b; SUNDA and GUILLARD,

1976; FISHER and FROOD, 1980). Nutrients may also influence the threshold of toxicity; for example, raising the concentration of silicon is reported to lower the threshold (MOREL et al., 1978). Altering the light-dark regime also has an effect (MANDELLI, 1969). WALSH et al. (1982) compared the effects of ten industrial wastes on *S. costatum* growth, and on *Selenastrum capricornutum* for reference. The two species responded rather similarly to each concentration of the different wastes tested and were inhibited by high concentrations. WALSH and GARGAS (1983) stated that the inorganic, particularly the cationic component, is more toxic than the organic. SANDERS and VERMEERSCH (1982) observed a decreasing in growth rate in the presence of arsenate from 54 to 70 % after addition of 5 to 25 $\mu g. l^{-1}$. The species can decline severely in natural waters receiving such toxic material. Boron at high concentration (30 $mg. l^{-1}$) plays a favourable rôle for growth, particularly in aged cultures. Improvement of photosynthesis in the presence of boron can reach 100 % according to SUBBA RAO (1981b). This author believes that boron in excess can relieve a nutrient deficiency.

S. costatum is also sensitive to cresols (petroleum compound), more so than diatoms of the genus *Chaetoceros* (THOMAS et al., 1980/1981). Its vulnerability to organic toxics (pesticides and industrial wastes) led WALSH and ALEXANDER (1980) to give an algal bioassay method which allows the use of *S. costatum* in rapid screening of these pollutants and even to associate it to animals in testing programmes for complex wastes (WALSH et al., 1980).

Much of the variability encountered with *S. costatum* may be ascribed to genetic differences. The remarkable studies by GALLAGHER (1980) may be cited : this author isolated 457 clones of *S. costatum* in the Narragansett Bay during [redacted] two-year period and studied the electrophoretic banding patterns of their allozymes. She found that summer bloom populations are genetically different from winter bloom populations. These genetic differences between seasons are [redacted] great as those recorded between species of vascular plants. Cells occuring during a bloom cannot be considered clones since several genetic varieties exist simultaneously, although some of the genotypes will become prevalent depending on the season. On the other hand, in spite of genetic variations, there is no noticeable morphological variations within the different genotypes. To this already complex situation, must be added the existence of heterozygotes and homozygotes in a same population, although loss of heterozygosity seems to occur with time during active growth and especially in cultures as mentioned by MURPHY (1978). GALLAGHER (1982) also carried out physiological studies among the preceeding clones and showed that the electrophoretic survey is not sufficient to estimate the actual genetic diversity. Under the same set of temperatures and light intensities, the range of growth rates among these clones was 0.1 - 5.0 div. d^{-1}; chlorophyll *a* concentrations and carbon uptake vary by a factor of *ca* 7 to 8. GALLAGHER et al. (1984) found that photoadaptation can take various forms : pigment ratios and photosynthetic unit sizes, for example, differ significantly from one clone to another. These results have important implications for ecology since they indicate that a single clone cannot

be entirely representative of the population it comes from. Moreover, the existence of numerous genotypes with different physiological characteristics can help to explain why ubiquitous species can develop in various hydrological conditions and why it is so difficult to foresee variations in abundance of *S. costatum* in natural waters (HULBURT, 1963).

GALLAGHER (1982) did not observe any important evolution in genetic patterns, the strains maintained in culture for months tending to present physiological similarities. This however may be due to culturing techniques : the clones were cultivated under extremely low light intensities and temperatures which led to very low division rates; such conditions, close to those of the stationary phase, lead more often to cell size restoration through vegetative enlargement than through sexual reproduction (GALLAGHER, 1982; 1983). More favourable conditions may increase sexual reproduction and thence selection of zygote lines with faster growth rates. MURPHY (1978) has already pointed out that such a selection in clonal cultures tends very quickly to produce homozygous populations, thus reducing the genetic information still further. It is perhaps vain therefore to expect any clonal culture to be physiologically or ecologically representative of the species and equally vain to expect uniformity between isolates. Standardization in the clones used in bioassays is therefore essential.

The question of standardization, however, does not arise if the assay requires the use of autochthonous representatives. Then several isolates must be made and used as soon as possible after isolation and conclusion should be based on a synthesis of the possibly very variable results. For assays requiring standard test organisms, ideally the same clone should be used by all workers rather than the plethora of mostly undocumented strains as present in use. As a compromise we suggest one of the three well documented and much used clones of *S. costatum* : (1) «SK6C» isolated in 1966 from Narragansett Bay and maintained by the Department of Oceanography, University of Rhode Island; (2) «No. 18» from Georgia Strait and maintained by the University of British Columbia and above all (3) «Skel» isolated from Long Island Sound in 1956 and maintained at Woods Hole Oceanographic Institution and also in various algal culture collections in Europe and North America. This last is the best known and the most used of all the cultures.

In conclusion, the great variability in behaviour both between and within clones, and in response of cell constituents to nutrient exhaustion, the variation in chain length and diameter not necessarily related to nutrient conditions all increase the burden of experimental protocol to the exclusion of precision and reproducibility. This applies to all chain-forming diatoms but especially to *Skeletonema costatum*. This species is however typical in inshore waters and frequently represents the major fraction of the biomass and it would be unwise to reject it from all tests. We simply note that in view of all the difficulties associated with *S. costatum* it should only be used as a standard assay organism if there is an overriding reason for doing so.

PHAEODACTYLUM TRICORNUTUM BOHLIN

First described by BOHLIN (1897) under the name by which it is now known and later by ALLEN and NELSON (1910) as *Nitzschia closterium* (Ehrenberg) Wm. Smith, forma *minutissima*, this alga has been in cultivation continuously for at least 80 years. WILSON (1946) first established the synonymy of the two descriptions.

There are four morphological forms of *P. tricornutum*, «fusifom», «triradiate», «cruciform» and «oval». Only the last of these are at all diatom-like in having one silicified frustule (LEWIN et al., 1958; LEWIN, 1958). The absence of silicon in the other forms and the complete absence of rafe had long placed in doubt the true taxonomic position (of BOURRELLY and DRAGESCO, 1955) and even now is considered to lie in a subclass of its own, since even in the «ovals» there is only one silicified valve.

The typical habitat is supralittoral rock pools, where it can occur in great numbers. The forms normally encountered in nature are the planktonic «triradiate» and «fusiform» forms, but the benthic «oval» form is likely to have been overlooked. The conditions governing the expression of the various morphological forms are still not clear. WILSON and LUCAS (1942) drawn the attention to the fact that, in cultures of the fusiform strain isolated by Allen and Nelson, proportions of triradiate cells increased from 1 to 99 % of the whole population within 10 years of maintainance. But WILSON (1946) observed that «triradiate» can also turn to «fusiform» or «oval» and their respective proportions vary according to culture conditions : triradiate cells occur more frequently in nutrient-rich media, which could explain why they usually dominate in cultures. However this form has frequently been recorded in nature and is not an artefact due to cultivation. Oval cells need good illumination and tend to dominate on solid media while fusiforms and triradiates develop better on liquid media (BARKER, 1935; LEWIN et al., 1958). According to BOROWITZSKA and VOLCANI (1978) genetic evolution as well as external factors (*e.g.* the presence of phytohormones) can induce morphological variations and COOKSEY and COOKSEY (1974) found calcium deficiency to induce the transition from «ovate» to fusiform cells.

Since in work published before 1960, *P. tricornutum* often appears as *Nitzschia closterium* (Ehrenberg) Wm. Smith forma *minutissima* and because *Nitzschia closterium* (Wm. Smith) is very different in its characteristics (LEWIN, 1958), whenever the «forma» is not included, the papers have not been taken into account in this review.

P. tricornutum has been much studied since this supralittoral species often predominates in cultures of natural populations. Nevertheless cell division is relatively slow for an organism of its size (*ca* 24 μm in length). SPENCER (1954), NELSON et al. (1979), SHARP et al. (1979), LI and MORRIS (1982), and TERRY (1983) mentioned maxima close to 2 div. d^{-1}. FAWLEY (1984) even recorded a maximum division rate of 2.16 div. d^{-1} at 23°C and BEN-AMOTZ and GILBOA (1980b) 2.40 div. d^{-1} at 10°C. GARGAS and PEDERSEN

(1974) and SUBBA RAO (1981a) mentioned maxima much lower, closer to 1 div. d^{-1}. Under natural conditions of light and temperature the rates are even lower (RAYMONT and ADAMS, 1958; ANSELL et al., 1963). NELSON et al. (1979) however note the that the rate is higher than that of *Thalassiosira pseudonana* at low irrandiance.

Temperature affects the growth rate up to 20°C (SPENCER, 1954). The maximum Q_{10} between 5 and 15°C is given as 2.5 by LI and MORRIS (1982) and as 2.3 by RAIMBAULT (1984). There is an inhibition of growth at 26.5°C (TERRY, 1983) and no growth is observed above 28-30°C (UKELES, 1961; LI and MORRIS, 1982; RAIMBAULT, 1984). In fact, as FAWLEY (1984) mentions, the effect of temperature on cell division rate depends on light intensity . For three light intensities (54, 117 and 208 $\mu E . m^{-2} . s^{-1}$) there is an increase in the number of divisions per day between 14 and 23°C and a decrease above 25°C; below 54 $\mu E . m^{-2} . s^{-1}$ the optimal temperature drops to 16°C at 7 $\mu E . m^{-2} . s^{-1}$. TERRY et al. (1983) estimated the irradiance compensation level at the low values of 5 and 6.3 $\mu E . m^{-2} . s^{-1}$ for their two strains of *P. tricornutum* grown at 25°C. Photoinhibition is observed at 550 $\mu E . m^{-2} . s^{-1}$, which is high compared to that of 160 $\mu E . m^{-2} . s^{-1}$ given by CHAN (1978) for diatoms (including *S. costatum*) cultivated at 21°C. From these various results it appears that *P. tricornutum* is well adapted to tolerate a wide range of light intensities. The strains studied by TERRY et al. (1983) divided more rapidly during the dark period. This observation differs from those given by PALMER et al. (1964) and UNO (1971) who noted increasing cell densities during the first half of the light period and by HUMPHREY (1979) who found more divisions during the second half of the light period. The discrepancies between these last results have to be ascribed to differences in the culture conditions and to the parameters these authors used to evaluate the frequency of divisions : optical densities or cell counts. In any case, it appears that differences recorded between light and dark periods are very likely slight.

GRIFFITHS (1973), while examining CO_2 assimilation, reported that incubation in darkness protects the cells' photosynthetic apparatus from high temperature injury. Usually cell chlorophyll *a* increases with increasing temperatures; productivity per unit chlorophyll *a* is maximum between 20 and 30°C (LI and MORRIS, 1982). But GOLDMAN and MANN (1980) found a carbon : chlorophyll *a* ratio constant whatever the temperature. GOLDMAN and RYTHER (1976) have shown that between 15 and 20°C *P. tricornutum* can efficiently outcompete neritic species such as *S. costatum* and *T. pseudonana* in outdoor mass cultures. On the other hand, NELSON et al. (1979) showed that, even at low light intensity, *P. tricornutum* is unusually able to sustain good cell division rates; and they added that «this efficiency may contribute susbtantially to its success in turbid, nutrient enriched mass algal cultures, the only environments in which it is known to attain great numbers».

This ability to adapt easily to environmental factors can be ascribed to carboxylation reactions. Indeed the important rôle of C_4 cycles in unicellular marine algae was first demonstrated with this species (BEARDALL and MOR-

RIS, 1975). This was confirmed in other diatoms, such as *S. costatum* by BEARDALL et al. (1976). It has a great ecological significance for it allows one to understand the mechanisms of photosynthetic adaptation to typical marine conditions (*i.e.* relatively high oxygenation, low light intensity, low carbon dioxide, high bicarbonate concentration at the usual seawater pH). In such conditions photorespiration is favoured in C_3 algae where carbon fixation mainly depends on ribulose-diphosphate-carboxylase (RUBPCase) activity. The adaptative capability of this so-called C_4 alga has to be attributed to an increase in the phosphoenol-pyruvate carboxylase (PEPCase) activity, according to MORRIS et al. (1978) or in the phosphoenol-pyruvate carboxykinase (PEP-carboxykinase) activity, according to KREMER and BERKS (1978) and DESCOLAS-GROS (1983). Adaptive capability can be an important factor in determining species successions in natural populations by giving a selective advantage to these C_4 algae.

Following MORRIS' (1980) idea one can visualize a wide spectrum of metabolic types, from Chlorophyta like *Dunaliella tertiolecta* with dominant C_3 enzyme activity to Dinoflagellates and Diatoms such as *P. tricornutum*, which show a mixed C_3 and C_4 photosynthesis. But the physiological states of the organisms can also modify the varying degrees of C_4 metabolism within a given alga : in batch cultures the ratio of RUBPCase : PEPCase activity decreases from the exponential growth phase to the stationary phase, which occurs when some factor becomes limiting.

Temperature appears to influence this ratio; LI and MORRIS (1982) found RUBPCase : PEPCase activity ratio varying from 12.2 at 5°C to 1.6 at 25°C, while, between 10 and 25°C, PEPCase activity remains constant while RUBPCase markedly decreases.

Cell volume in *P. tricornutum* varies very little with culture conditions. GARGAS and PEDERSEN (1974) quote a range of 38.5 to 59.9 μm^3. More variation however would be expected in the longer term, associated with a possible change in the growth form («fusiform», «triradiate», «oval»). BROWN and RICHARDSON (1968) observed that cells grown at 25°C varied in volume with light intensity, the largest cells occurring for the low light intensities, whereas FAWLEY (1984) does not observe such changes in cell size, but records slight variations of cell width with temperature (the width increasing at high temperatures (23 and 25°C) and the length remaining unaffected). *P. tricornutum* is therefore well suited to electronic particle counting notwithstanding the fact that «triradiates» and «fusiforms» (but never «ovals») do very occasionally form chains (COUGHLAN, 1962). Such chains have not been observed in nature (BOROWITZKA et al., 1977) nor in mass cultures (ANSELL et al., 1964). But when maintaining cells in laboratory cultures, Borowitzka et al. found chains of up to 200 cells firmly attached to each other at the central region of their theca, that were difficult to break up by stirring or sonication.

P. tricornutum is very euryhaline, more so than *S. costatum*. BERLAND (1966) quoted a range of 2.5 to 72 ‰ and HAYWARD (1968c) 4.4 to 87.5 ‰.

Furthermore. BRAND (1984) found noticeable growth (from 0.35 to 1.24 div. d^{-1}) at 0 ‰.

Much will depend on the previous history of the clone but DROOP (1958a) found maximum cell count to be obtained at salinity between 2.5 and 18 ‰ whilst CHIAUDANI and VIGHI (1978) obtained maxima between 12 and 36 ‰ but with a small diminution in cell volume at lower salinities. HAYWARD (1970) reported lower division rates at 24 ‰ than at 12 ‰. According to DROOP (1958a) the difference in salinity tolerance between *S. costatum* and *P. tricornutum* and other very euryhaline algae can be ascribed entirely to the latter's comparatively greater indifference to the Na ion concentration *per se*.

The nutrition of *P. tricornutum* is comparatively well known. The requirements for nitrogen and phosphorus were the subject of the now classical studies of KETCHUM (1939) and KUENZLER and KETCHUM (1962), which first drew attention to the phenomenon known as «luxury consumption». They found than when inocula were placed in fresh culture medium virtually all the phosphorus was taken up within a few hours so that the cells became replete. Growth of the alga in the days that followed took place independently of concentration of the phosphorus in the medium while the internal concentration of cell phosphorus decreased exponentially to a certain minimum, whereupon growth ceased. The physiological and ecological implications of this are very important and also concern other algae and other nutrients (*e.g.* DROOP, 1973, 1974). But from a bioassay point of view the fact that the amount of growth depends on the total rather than merely the external nutrient emphasizes the need to standardize the depletion of inocula.

Nitrate and nitrite uptake have been studied by CRESWELL and SYRETT (1979, 1981, 1982). They demonstrated that cells lack ability to take up these nutrients when ammonium is present in high concentration, but acquire the ability after a period of nitrogen deprivation. The absorption of the two nutrients shows numerous similarities, which lead the authors to conclude that the mechanisms on which these processes depend are the same. When ammonium, nitrate and nitrite are present together, *P. tricornutum* takes up NH_4 first and then NO_3, nitrite being absorbed only when the two other ions have completely disappeared from the medium.

The process becomes more complicated when the interactions between nitrate and/or ammonium and other ions, such as phosphate, in the light and in the dark are taken into account. Thus TERRY (1982b) confirms that the uptake rate for nitrate is reduced by addition of ammonium to the medium, as demonstrated by Syrett and coworkers, but adds that it is also reduced by addition of phosphates, which argues for an involvement with photosynthesis. However *P. tricornutum* can fix phosphorus in the dark, so there is no direct relationship between photosynthetic activity (and CO_2 fixation) and phosphorus uptake (TERRY, 1982b), contrary to what have been found by CHISHOLM and STROSS (1976) for other algae and by HARRISON et al. (1977b) for natural phytoplanktonic communities. On the other hand, the assimilation of nitrate-N needs a high level of energy probably derived from respiratory and

photosynthetic reactions (a similar observation was made for *S. costatum* by BATES, 1976). As a result, the increase of NO_3-N assimilation in the light is accompanied by a relative decrease in CO_2 fixation. According to TERRY (1982b) it is logical to admit that the inhibiting effect of PO_4 on the NO_3 uptake is incomplete because phosphate has no effect on the reduction and assimilation processes themselves and only competes with the nitrate uptake system in the dark. Then uptake of NO_3 and PO_4 competes for the ATP produced by oxidative phosphorylation. TERRY (1983) also finds a competition between ammonium and phosphate uptake. If the two ions are added simultaneously to a culture of *P. tricornutum* the uptake rates for the two ions are lower than those observed if the two ions are added separately. Moreover, in the light, ammonium inhibition of phosphate uptake is greater (46 %) than phosphate inhibition of ammonium uptake (15 %). In the dark, there is little difference (30 and 31 % inhibition respectively). The difference between the light and the dark behaviour can be ascribed to the fact that «phosphate uptake competes on an approximately equal basis with ammonium uptake for energy produced by oxidative phosphorylation, while ammonium uptake is the superior competitor for ATP produced by photophosphorylation».

P. tricornutum has considerable heterotrophic ability, although in our experience and that of HAYWARD (1968a) it is an obligatory phototroph, being unable to utilize energy from such organic compounds as sugars, fatty acids and amino acids for dark growth (see however COOKSEY, 1974, who reported some dark growth on acetate). It is probable that some carbon substrates can be assimilated phototrophically. In contrast, the range of organic compounds able to satisfy the phosphorus and nitrogen requirements is comparatively great. The production of alkaline phosphatase under P-starvation (KUENZLER and PERRAS, 1965) is such as to enable a quick hydrolysis of organic P excreted by other microorganisms. The range of amino acids that can be utilized in high concentration is great (RYTHER, 1954; HAYWARD, 1965; ANTIA et al., 1975). WHEELER et al. (1974) found 8 of the 9 amino acids they studied to be taken up even at very low concentrations. LU and STEPHENS (1984) demonstrated that *P. tricornutum* is able to remove the 13 amino acids they tested, very fast from sea water even at the low concentration of 0.2 μM. The percentage of amino acid removed in 60 minutes varies between 55 and 100 %.

P. tricornutum is able to take up urea by an active mechanism. This uptake can occur in darkness but is markedly stimulated by light. The affinity is high, the half-saturation constant being about 1 μM. Presence of ammonium appears to suppress the urea transport system (REES and SYRETT, 1979). SHAH and SYRETT (1982) also demonstrated that nitrate-cultured cells of *P. tricornutum* can take up guanine after nitrogen deprivation with a half-saturation constant of 0.48 μm. But in these studies the amounts of nutrients used were always very great and very different from the biotopes from which *P. tricornutum* had been isolated. As a result, their data cannot reflect the true nutritional capacities of the species in the natural environment. Another peculiarity of *P. tricornutum* is that its need for silicon is much lower than is normal in diatoms (0.3 % compared to 14 % in *e.g. S. costatum*, PARSONS et al., 1961). This is

accounted for by the complete absence of silicon from the «triradiate» and «fusiform» forms and only a small amount in the «ovals» (LEWIN et al., 1958). Recently NELSON et al. (1984) showed that half-saturation constant of Si uptake is much higher in *P. tricornutum* (97.4 µM) than in diatoms forming siliceous frustules (1 to 6 µM). In nature, *P. tricornutum* takes up Si without an apparent requirement but is kinetically incapable of strongly competing for Si with other diatoms that absolutely require it for growth. The low requirement for silicon should be an advantage in littoral waters where other nutrients are often very high (D'ELIA et al., 1979).

P. tricornutum has no requirement for exogenous vitamins (DROOP, 1958b); but it has been observed to take up vitamin B_{12} just like auxotrophs (DROOP, 1968) and to excrete thiamine (CARLUCCI and BOWES, 1970b). The requirement for iron is lower than for less neritic species (DROOP, 1962; HAYWARD, 1968b). As in other species, iron deficiency is accompanied by a reduction in cell chlorophyll *a* (HAYWARD, 1968b) and in protein and cytochrome f (GLOVER, 1977).

According to BERLAND et al. (1976) *P. tricornutum* is one of the least sensitive algae to heavy metal pollution. It will tolerate up to 25 000 µg Zn. l^{-} without any decrease in division rate, in contrast to *S. costatum* and *T. pseudonana* where the limits are respectively 25 and 250 µg. l^{-1} (JENSEN et al., 1974). With copper, JENSEN et al. (1976) quote ■ limit of 400 µg. l^{-1} (10 and 25 µg. l^{-1} for *S. costatum* and *T. pseudonana* respectively). However CHIAUDANI and VIGHI (1978) quote the lower figure of 100 µg Cu l^{-1} for *P. tricornutum*. BRAEK et al. (1976) found the interaction between copper and zinc to be mutually antagonistic for *P. tricornutum* (but synergistic for *S. costatum, T. pseudonana* and *Amphidinium carteri*) with the result that mixtures of the two metals of up to 4000 µg Zn. l^{-1} and 250 µg Cu. l^{-1} do not affect growth of *P. tricornutum*. Numerous chelators can occur in natural conditions, especially in coastal waters rich in seaweeds. Thus RAGAN et al. (1980) showed that exudation of polyphenols from *Ascophyllum nodosum* and *Fucus vesiculosus* contribute to the natural chelating capacity of the water since they relieve the toxicity of Zn to *P. tricornutum*. Furthermore, HAWKINS and GRIFFITHS (1982) showed that *P. tricornutum* exposed to copper for three days is capable of recovering from concentrations in metal strongly inhibitory to cell division after a lag period of three more days. This capability of recovery disappears when exposure time increases : for an exposure time of 10 days, growth is completely inhibited.

KUSK (1981) measured the variable sensitivity of *P. tricornutum* towards crude oil extracts and aromatic carbons. The responses of the alga depend not only on the nature and concentration of the pollutant but also on culture conditions and more especially on light intensity.

LUNDY et al. (1984) knowing the sensitivity of this species, used it in a double algal bioassay in association with *T. pseudonana* for determining the toxicity of other pollutants. They exposed the two algae together to different chemical products and found that, in their experimental ecosystem, DDT at

the concentration of 10 ppb (10^{-9}) significantly altered the species ratios in favour of *T. pseudonana* while the same quantity of polychlorinated-biphenyl (PCB) produced an alteration in favour of *P. tricornutum*. Competition experiments between *D. tertiolecta* and *P. tricornutum* have also been carried out by GOLDMAN et al. (1982b). The frequent domination of *P. tricornutum* comes from its tolerance of the very alkaline conditions (pH > 10) occurring in intensive but irregularly controlled cultures (GOLDMAN et al., 1982a). The ability of *P. tricornutum* to outcompete increases with increasing pH; hence pH control may be a method for maintaining the dominance of species other than *P. tricornutum* in mass cultures.

P. tricornutum is thus comparatively insensitive to modifications in its environment, either physical, *e.g.* light and temperature, or chemical, *e.g.* silicon and growth factors, or pollutants. This species is therefore the best suited to measuring the total concentration (mineral or organic) of the major nutrients in AGP measurements. This is particularly so since final biomass and initial nutrient titre have been shown to be linearly related up to quite high concentions (SPENCER, 1954; GARGAS and PEDERSEN, 1964; CHIAUDANI and VIGHI, 1978). GARGAS and PEDERSEN, moreover, note that this is so whether the measurement is cell count, population volume or dry weight. The species is also well suited to electronic counting.

To qualify as a tool for bioassay, an alga must be easily maintained in routine cultures without too many replications, *i.e.* have a low growth rate. One way to diminish the growth rate is to lower the temperature. AIZDAICHER and SILKIN (1983) studied the viability of *P. tricornutum* maintained in darkness at 22°C, 8°C and − 5°C with glycerin and dimethylsulfoxide as substrate or cryoprotector in the medium. From the results, which are complex, it appears that the highest cell viability is observed at 8°C. Glycerin increases cell preservation and at − 5°C is used as a cryoprotector. BEN-AMOTZ and GILBOA (1980a) improved the cryopreservation process by using a two-step freezing technique (cells suspended in 5% dimethylsulfoxide at 20°C for 15 minutes then cooled at −30°C for 15 more minutes and finally frozen in liquid nitrogen). In these conditions there were 29% recovery. This large recovery after storage is advantageous in a test alga.

There are many strains of *P. tricornutum* in existence, with little to choose between them (HAYWARD, 1968c; TERRY et al., 1983). We suggest that the oldest and indeed most utilized strain, namely No 1052/la of the Cambridge Collection, is to be preferred over others for bioassay work.

THALASSIOSIRA PSEUDONANA (HUSTEDT) HASLE et HEIMDAL and *THALASSIOSIRA OCEANICA* HASLE

Originally known as *Cyclotella nana* Hustedt, *Thalassiosira pseudonana* (Hustedt) Hasle and Heimdal (HASLE and HEIMDAL, 1970) was first described from freshwater (HUSTEDT, 1957). However it has since been recorded

from many marine localities and there has only been one recent recording from fresh water (BELCHER and SWALE, 1977). BRAND et al. (1981) have suggested that the comparatively recent cosmopolitan appearancies of this organism, particularly in neritic waters, is mainly due to human influences (*e.g.* transport in ship's ballast and use in aquaculture) aiding the spread of a particularly tolerant form of the species.

In their pioneering study at Woods Hole, GUILLARD and RYTHER (1962) used 5 isolates taken from widely differing habitats in 1958. Clones «3H» and «5A» from neritic waters of Moriches Bay, New York; clone «e.p.» from a littoral lagoon; clone «7-15» from the edge of the continental shelf; and clone «13-1» from the Sargasso Sea.

Referring to general morphological criteria, HASLE (1978) described clone «7-15» as a new species, *T. guillardi*, and later on clone «13-1» as a separate species under the name of *T. oceanica* (HASLE, 1983). In early papers authors only referred to *Cyclotella nana* or *Thalassiosira pseudonana* without noting the clones they used. «13-1» and «3H» became the clones more frequently studied and subsequently will be the ones of concern to us here. But in order to avoid confusing nomenclature in the following text, clone designations when known will be given in place of species appellations. It soon became apparent that there were distinct differences between the two clones, though their dimensions in culture are rather similar : 2.5 to 10 μm diameter as against 4 to 11 μm (GUILLARD and RYTHER, 1962) and 85 to 140 μm^3 cell volume for «3H» and «13-1» respectively (KUENZLER and PERRAS, 1965). HITCHCOCK (1982) gave similar information for «3H» with 97 μm^3 but HEATH (1982) found slightly lower values, between 50 and 65 μm^3. The differences are greater in the field : 2.5 to 4 μm mean diameter for neritic and *ca* 10 μm for oceanic populations. HALLEGRAEFF (1984) identifying 25 taxa belonging to the genus *Thalassiosira* from Australian waters gives 3-4 μm and 4-10 μm for *T. pseudonana* and *T. oceanica* respectively.

One has to be cautious in comparing division rates for species of the genus *Thalassiosira* because these rates can vary greatly depending on the cell status respecting auxosporulation. Thus COSTELLO and CHISHOLM (1981) found in *T. weissflogii* (Grunow) Fryxell et Hasle (= *T. fluviatilis* Hustedt), species close to «13-1» and «3H», a twofold increase of the division rate when small-sized cells turn to large-sized cells after auxosporulation.

«3H», the neritic strain, is more euryhaline than the other. For example the growth rate only increases from 2.8 to 3.4 div. d^{-1} between salinities of 0.5 and 32 ‰, whereas «13-1», the oceanic strain, will not grow in salinities below 16 ‰ and even is sometimes difficult to maintain in culture below 26 ‰ (GUILLARD and RYTHER, 1962). BRAND (1984) found «3H» reproduction rates not to be reduced even at salinity of 45 ‰ but specimens can also be recorded in lakes and rivers.

Similarly, there is a difference in the response to temperature. GUILLARD and RYTHER (1962) mentioned that «13-1» is clearly a warm-water form as it does not survive when transferred at 10°C. They quoted for this strain 1 div.

d^{-1} at 15°C and more than 1.8 at 20°C and 24°C. RAIMBAULT (1982) also recorded no growth at 10°C, as well as at 30°C, and found 0.1 div. d^{-1} at 12°C, 0.5 at 15°C and 1.44 at 25°C. He gave a Q_{10} between 15 and 25°C of 2.7. BRAND et al. (1981) quoted a division rate varying from 0 at 12° to 3 at 24°C. In contrast, for «3H» GUILLARD and RYTHER (1962) observed a rate of 0.25 div. d^{-1} at 4° and between 2 and 2.6 div. d^{-1} at temperatures between 10 and 25°C, almost similar to that of *ca* 2 given by SHARP et al. (1980) at 18°C. However, BRAND et al. (1981) found higher values, from 1.7 div. d^{-1} at 12°C to more than 4.5 at 24°C. In the particular conditions of cultures in dialysis sacks immersed in a Norwegian fjord, HEGSETH and SAKSHAUG (1983) mentioned for «3H», division rates of *ca* 0.2 div. d^{-1} in December and February when temperatures lay between 4.5° and – 0.9°C and the light intensity was particularly low. There is no longer any doubt that «3H» is a very eurythermal strain, which makes it an effective competitor at extreme and more especially at higher temperatures.

According to GOLDMAN and CARPENTER (1974) clone «13-1» obeys the Arrhenius law as do the majority of diatoms, whereas «3H» does not. But BRAND et al. (1981) did not observe such a difference : for the 14 clones they studied (among which «13-1» and «3H») they noted a nearly linear increase of growth rates with increasing temperature. Furthermore, very small differences are observed among clones from neritic waters around the world while much greater ones are noted among oceanic clones. In continuous culture, «3H» shows little difference in production rate of cell carbon over a large temperature range (10° to 30°C), unlike some other neritic species (GOLDMAN and RYTHER, 1976). One would predict that in competition neritic *Thalassiosira* would outgrow *Phaeodactylum tricornutum* at both low (10°C) and high (25°C) temperatures while *P. tricornutum* would win at intermediate temperatures. Irradiance would also affect the competition since «3H» has the higher growth rate at high incident irradiance (NELSON et al., 1979).

RYTHER and GUILLARD (1962b) also found that the effect of temperature on its respiratory coefficient was generally much higher and more temperature-dependent than that with «13-1», which is in accordance with general experience of eurythermy among the more neritic and stenothermy among the more oceanic species. MORRIS (1980) studied the carboxylating enzymes in the two strains. The RUBPCase : PEPCase ratio was always higher in «13-1». The high PEPCase activity in «3H» was considered to be an adaptation to the disequilibration *vis-à-vis* nutrients and light encountered in the neritic habitat. Other biochemical differences have been observed between the two strains; for example MURPHY and GUILLARD (1976) examined the electrophoretic characteristics of enzymes from several clones of species belonging to the genus *Thalassiosira* and found large differences between neritic (including «3H») and oceanic (including «13-1») strains.

BRAND and GUILLARD (1981) studying the effects of light on marine phytoplankton tested four intensities (0.01, 0.023, 0.1 and 0.23 ly. min^{-1}, *i.e.* *ca* 32, 75, 325 and 750 μE. m^{-2} . s^{-1} according to conversion factors given by

STEEMANN NIELSEN (1975) and HARRIS (1978)) and found that among strains of the genus *Thalassiosira*, clone «3H» from neritic area reproduces more rapidly in continuous light than in the 14:10 light-dark cycle. By contrast, a *Thalassiosira* sp. (clone A 615) from an oceanic area reproduces more slowly in continuous light than in the light-dark cycle. According to these authors one of the possible explanations for the different behaviour of these two clones *vis-à-vis* the light regime may be that they were used to particular light regime specific of their originating habitat. For many reasons (given by Brand and Guillard), in coastal regions, phytoplankton may experience light fluctuations in time scales much shorter than a day, while oceanic species experience a light regime strongly dominated by the diurnal cycle. The latter organisms would be the more sensitive to continuous light. It has been observed (NELSON and BRAND, 1979) that oceanic and neritic strains differ in the rhythms of cell division. The latter (*e.g.* «3H») could be trained to the same 14:10 light-dark cycle with cell division occurring 4 to 6 hours after the onset of the light period, whereas the oceanic strain remained unsynchronized. It was suggested that a resistance to synchronizing influences would be an advantage in an oligotrophic environment.

When measuring *in vivo* chlorophyll fluorescence in numerous species, BRAND (1982b) observed that «3H» is a strain capable of maintaining persistent diel rhythms in continuous light despite a reproduction rate greater than 1 division per day. This is contrary to the hypothesis given by EDMUNDS and ADAMS (1981). After BRAND (1982b) this capability may be common among phytoplankton species whose behaviour, related to an internal biological clock, could be independent of environmental factors for much longer than has previously been thought possible. BATES and PLATT (1984) using clone «3H» and *Dunaliella tertiolecta* stated that *in vivo* chlorophyll fluorescence induction can be used as a good index of photosynthetic capacity in marine phytoplankton, saving time usually required for classical ^{14}C incubation measurements. With the neighbouring species *T. weissflogii*, BIENFANG et al. (1983) demonstrated that sinking rate is slower at low light intensities, *ca* 0.10 m. d^{-1} for 13 to 27 $\mu E.\ m^{-2}\ .\ s^{-1}$ and 0.20 to 0.30 m. d^{-1} for 65 to 847 $\mu E.\ m^{-2}\ .\ s^{-1}$. As a result, in natural conditions, accumulation of diatoms occurs at low light intensities, which, added to an increase in cell chlorophyll concentration, can explain the existence of a subsurface chlorophyll maximum in temperate waters.

CHISHOLM et al. (1980) formulated the hypothesis that strict phasing in a cellular event, such as division occurs in diatoms when the cells are subjected to periodical variations of the factor limiting growth. This stimulus can be different from light. More precise work to this end has been carried out by CHISHOLM and COSTELLO (1980), OLSON and CHISHOLM (1983) and WHEELER et al. (1983), but again on *T. weissflogii*. They demonstrated that nutrient pulses interact with photocycles in the timing of cell division. YODER et al. (1982) added that influence of the nutrients depends on the way they are supplied : if provided once a day, nitrate does not modify the periodicity of cell division, whereas if provided at 2 h intervals, cell division occurs continuously throughout the 24 h.

Marked differences have also been observed in the nutritional characteristics of the two clones. CARPENTER and GUILLARD (1971) quote a K_s for NO_3-N uptake of 1.87 μg-at. l^{-1} for «3H» and 0.38 μg-at. l^{-1} for «13-1». Previously, EPPLEY et al. (1969) had obtained a similar figure for NO_3-N and between 0.4 and 0.5 μg-at. l^{-1} for NH_4-N in clone «13-1». These K_s values are however liable to be influenced by the previous history of the culture as CAPERON and MEYER (1972b) demonstrated. With «13-1» these authors obtained values ranging from 0.18 to 0.54 μg-at. l^{-1} for the K_s for NO_3-N. Similarly EPPLEY and RENGER (1974), in a comprehensive chemostat study of clone «13-1», found the K_s for both NO_3 and NH_4 to decrease with increasing N starvation. However the K_s variation observed within this clone has always been less than the difference between the two clones. PARSLOW et al. (1984a; 1984b) studied the transient responses of clone «3H» to ammonium or nitrate starvation. They found that cultures starved in ammonium developed very high specific uptake capacities due to both decreases in cell quota (to less than 10 %) and increases in uptake rates (up to 500 %). On the contrary, the specific uptake rates of nitrate-starved cells, growing on nitrate or on ammonium, can change with depletion time but are always low compared with those of ammonium-starved cells growing on ammonium. This may be mainly explained by the relative large cell quotas in nitrate-starved cells. DORTCH et al. (1982) had already pointed out that these different patterns of response seem to correspond to an adaptation of phytoplankton cells to the nutrient availability in natural waters : ammonium, like phosphorus has a higher turnover rate compared with nitrate and silicate which are usually recycled at much longer time scales in sea water.

With urea and uric acid GUILLARD (1963) found growth of «3H» to be 6 % of that observed on nitrate, while that of «13-1» was very poor. McCARTHY (1971) did not find any difference between the two clones respecting urea. Whatever the truth, the affinity for urea is comparatively great (K_s = 0.42 μg at. l^{-1}), and growth rates upon it are of the same order as those obtained with NO_3 or NH_4. HORRIGAN and McCARTHY (1981) demonstrated that «3H» is able to take up urea very rapidly, even when adequately supplied with NO_3-N or NO_2-N. The initial instantaneous uptake rate is far in excess of uptake rate required for cellular growth whatever the nitrogen nutrient status (predepletion, at depletion, postdepletion) and the maximum instantaneous uptake rate appears to be the same whatever the status. But HORRIGAN and McCARTHY (1982) added that the presence of NH_4 in the medium decreases the enhanced ability to take up urea. The ability to utilize urea as nitrogen source had also been found in *T. fluviatilis (= T. weissflogii)* by CONOVER (1975). By contrast, no amino acids support growth of clone «3H» (WHEELER et al., 1974); data on «13-1» are wanting.

T. pseudonana has been the subject of a number of papers on phosphorus limitation (FUHS, 1969; FUHS et al., 1972; CANELLI and FUHS, 1976). Although the maximum growth rate depended on light and temperature in the chemostat, it was always obtained with a very low phosphorus concentration (*ca* 1.0 μg-at. l^{-1}). The K_s for uptake according to PERRY (1976) was the same for both clones, namely 0.7 μg-at. l^{-1}. KUENZLER and PERRAS (1965) report

a rather low production of alkaline phosphatase, with that of clone «13-1» tenfold that of «3H». Maximum acitivty was observed at the very high pH of 9.8.

PARSLOW et al. (1984b) mention that the response of clone «3H» to phosphate starvation shows stronger patterns than those observed for ammonium response. Under phosphate starvation, cell division continues for a long time and the phosphate cell quota can strongly decrease; consequently the specific uptake rate measured in perturbation experiments shows a dramatic increase during the starvation period. For example, it exceeds sixty times μ max (maximum specific growth rate) after 48 h starvation. PARSLOW et al. from these observations point out that clone «3H» could double its cell P quota each day if exposed to saturating phosphate concentrations for just a few minutes a day. As for NH_4 these elevated transient uptake rates can be interpreted as an adaptation to microscale nutrient patches generated by zooplankton excretion (as developed by McCARTHY and GOLDMAN, 1979, and LEHMAN and SCAVIA, 1982).

PAASCHE (1973b) studied the silicon requirement of the clone «3H». He measured a half-saturation constant (K_s) for growth ranging from 0.5 to 0.8 μg-at. l^{-1} Si and a maximum growth rate from 2.44 to 2.73 div. d^{-1}. GUILLARD et al. (1973) found that «3H» has a higher K_s (0.98 μg-at. l^{-1} Si) and a higher maximum (3.6 div. d^{-1}) than «13-1» (K_s = 0.19 μg-at. l^{-1} Si and maximum of 2.1 div. d^{-1}). PAASCHE (1973c) also recorded a K_s for uptake ranging from 0.91 to 2.13 μg-at l^{-1} for «3H», *i.e.* intermediate between the lower values of *Skeletonema costatum* and the higher ones of other diatoms, *Licmophora* sp., *Ditylum brightwellii*, *Thalassiosira decipiens*. NELSON et al. (1976) confirmed Paasche's K_s uptake values for «3H» (between 0.8 and 2.3 μM) and for «13-1» quoted similar values, but in a narrower range (1.4 to 1.5 μM); they found the neritic strain to have a maximum specific uptake rate three times higher than that of the oceanic strain (0.062 - 0.092 pg Si $cell^{-1}$ h^{-1} as against 0.028 - 0.031). These results for «3H» are very similar to those of PAASCHE (1973) who gave a mean value of 0.073 pg. Si $cell^{-1}$. h^{-1} for V_{max}.

Both clones are auxotrophic with respect to vitamin B_{12} (GUILLARD and RYTHER, 1962), but they differ in their ability to utilize the vitamin B_{12} analogues (GUILLARD, 1968), «3H» having virtually «mammalian» and «13-1» «coli» specificity. The specificity and great sensitivity of «3H» recommend it for vitamin B_{12} assay (RYTHER and GUILLARD, 1962a; SWIFT, 1984), and especially for the vitamin proper (SWIFT and GUILLARD, 1977). However it should be pointed out that few kinetic data exist on the vitamin B_{12} requirement of the two clones.

Thalassiosira is very sensitive to heavy metals. BERLAND et al. (1976) record effects on growth of concentrations of 10 μg. l^{-1} copper or cadmium, 5 μg. l^{-1} mercury and 250 μg. l^{-1} lead. Clone «3H» was the most sensitive of 18 phytoplankters tested. As was mentioned previously, the response of algae to heavy metals is very dependent on the physico-chemical condition of the medium. Thus MANDELLI (1969) reports for «13-1» a high toxic threshold of 230 μg. l^{-1} for copper in a medium containing the chelating agent EDTA, while ERICK-

SON (1972), with the same clone and using filtered but otherwise untreated water, found the threshold to lie between 0.68 and 6.14 $\mu g. l^{-1}$ Cu. Since then SUNDA and GUILLARD (1976) have shown clearly that toxicity is a function of the free metal ion activity, which in turn is salinity-, pH- and chelator-dependent (DROOP, 1961b). The mass action laws predict that quite small differences in concentration can result in large differences in activity and consequently on algal growth rate, as has been demonstrated by GAVIS et al. (1981). Several authors investigated the mechanisms of the Cu action in the genus *Thalassiosira*. RUETER (1983a) demonstrated with *T. weissflogii* that, for a given cupric ion activity, the Cu toxicity decreases when the soluble pool of silicic acid increases in the medium. At sublethal activities, this toxicity introduces modifications in frustule shape and size of clone «3H», due to interference with the processes of silicon deposition (THOMAS et al., 1980b; RUETER et al., 1981). RUETER and MOREL (1981) studying actions of Cu and Zn on silicon uptake, concluded that it was the Cu:Zn ionic ratio that affected Si uptake. RUETER et al. (1981) found no toxic effect of Cu on NO_3 and PO_4 uptake but RUETER (1983b) showed an inhibition of alkaline phosphatase activity, which may reduce the alga's ability to use organic phosphorus as an alternative source of phosphorus. SUNDA and HUNTSMAN (1983) demonstrated that there is a competition between Cu and Mn: Cu blocks cellular Mn uptake or binding within intracellular pools, causing Mn to become limiting and growth rate to decrease.

Another type of antagonism between metals is described in *T. weissflogii* by ANDERSON and MOREL (1982), FOSTER and MOREL (1982) and HARRISON and MOREL (1983). Cadmium toxicity is reversible and the reversal of toxicity is modulated by iron concentration in the medium. For high ionic Cd:Fe concentration ratio, the uptake rates of Fe are low, which leads to a cytochrome depletion, to a large decrease of the Fe-dependent enzymes such as nitrate reductase and finally to the lowering of growth. When Cd and Fe ionic concentrations are both high, uptake rates of Fe increase and cell can accumulate iron in excess and growth is better, though not optimal. According to HARRISON and MOREL (1983) in these latter conditions, cells appear to remain Fe-deficient despite their high intracellular iron concentrations. The authors hypothesize that cadmium, not only competes with Fe for uptake sites but also blocks its intracellular utilization. FOSTER and MOREL (1982) suggest that the antagonistic effect of iron at sufficient concentration *vis-à-vis* heavy metals, could explain the resistance of phytoplankton to the negative action of industrial wastes in the natural environment. Another type of interaction can be put forward to explain the results of FREY et al. (1983) who tested the effects of chromium in the anionic hexavalent form on clone «3H» over a wide range of salinities. It appears that chromium toxicity varies according to the salt strength of the medium. With 3.8 μM Cr no growth occurs at the salinity of 0.03 ‰, whereas it is possible to record significant growth at 1.1 ‰ and at 2.1 ‰ the inhibiting effect of the metal is neutralized. RIEDEL (1984) demonstrated why the toxicity of Cr depends on salinity : the inhibition by Cr is a function of the ratio of Cr to sulfate and inhibition occurs when this ratio exceeds *ca* 500:1. As previously recorded with other metals, the author suggests

that the mechanism of lowering the Cr toxicity involves sites for which sulfate and chromate ions can compete. In contrast, there has been observed synergistic toxic effects between Cu and Zn (BRAEK et al., 1976) and between Cd and Zn (BRAEK et al., 1980) on growth of the same clone «3H». It appears now that the interaction between ions and their effect on growth are much more complex than initially expected. They depend not only on ion activities but also on concentrations of the major nutrients, light intensity and the preconditioning of cells. The existence of so many factors can explain why one can find varying responses to metals in the same strain. Marked differences have also been found between the responses of clones «3H» and «13-1» to metal ion activities. GAVIS et al. (1981) and GAVIS (1983) tested increasing concentrations of copper on marine phytoplankters. They reported that «13-1» was killed by a *p*Cu of 9.8, whereas the lethal concentration for «3H» was more than ten times higher at 8.5. The resistance of the clone «3H» can be related to its presumably metal-polluted estuarine origin.

At low concentration, several metals can also become limiting for growth. BRAND et al. (1983) gave results for iron, manganese and zinc. With no Fe added to the culture medium (natural water from Sargasso Sea), «13-1» grew at 1.45 div. d^{-1}, quite similar to that obtained with 10^{-9} M Fe. On the other hand, «3H» does not grow until the Fe concentration reaches 10^{-9} M (0.52 div. d^{-1}), and the maximum division rates (1.94 and 1.81 div. d^{-1}) occurred at Fe concentrations of 10^{-6} and 10^{-7} M Fe respectively. Similarly growth of «13-1» is three times faster than that of «3H» in a medium without added Mn or Zn. The maximum division rates occur at different metal concentrations : 10^{-8} M (Mn or Zn) for «13-1» and 10^{-6} M (Mn or Zn) for «3H». SUNDA and HUNTSMAN (1983) identified the adaptation allowing clone «13-1» to grow at low Mn concentration as a great ability of its cells to take up the metal and a low cellular Mn requirement for growth.

MURPHY and BELASTOCK (1980) have found «3H» to be more resistant than «13-1» to pollutants associated with industrial waste (mainly methylsulfate). But «3H» is more sensitive than «13-1» to polychlorinated biphenyls (FISHER et al., 1973; LUNDY et al., 1984). As previously mentioned *à propos P. tricornutum*, in a DDT-polluted medium, clone «3H» is less affected than *P. tricornutum* but more affected in a PCB-polluted medium.

Having regard for the many physiological differences between them, the two clones should be treated as two quite distinct entities, at any rate for the purpose of bioassay. Clone «13-1» could be used for comparatively poor ocean waters while «3H» would be suitable for neritic waters. Clone «13-1», because of its sensitivity to heavy metals, would be especially useful in combination with a less sensitive species, such as *Dunaliella tertiolecta*, for assessing the influence of heavy metals on AGP. Because of the existence of a diploid vegetative state and a capacity of self-fertilization, *T. pseudonana*, like *Skeletonema costatum*, has tended to become progressively less heterozygous and consequently less variable in culture (MURPHY, 1978). Both cultured strains are more than 20 years old and so cannot now properly be considered representative

of the populations from which they were isolated. On the other hand, their acquired genetic stability renders them very suitable for comparison of bioassay results over space and time.

A good knowledge of the physiological characteristics of this alga and its ability to withstand wide environmental variations renders it a potentially useful food organism for invertebrate larvae in the aquaculture industry. Researches have been undertaken to improve the yield of the alga (NEY et al., 1981) and to balance the carbohydrates : proteins ratio (GALLAGER and MANN, 1981/1982).

We suggest that *T. pseudonana* could be used with profit in all marine bioassay work. It has much to offer from a practical point of view : (1) it is easy to handle; (2) chlorophyll *a* variation with nutrient conditions, though sizeable, is less than with *S. costatum*; (3) the chains are short and easy to break up for electronic cell counts; (4) the growth rate can be very high; and (5) both clones «31-1» and «3H» are well known physiologically.

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– limiter à 5 mm au maximum les intervalles entre les photographies;
– éviter les réductions pour une meilleure qualité de reproduction; en conséquence, les dimensions des planches ne devraient pas dépasser 11,5 cm x 17,5 cm;
– pour les illustrations en couleur, demander l'accord de la Rédaction.